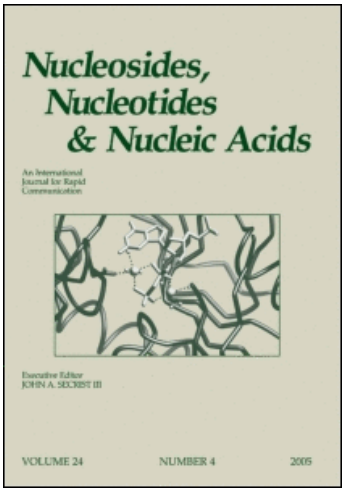

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Combinatorial Library of Artificial Ribonucleases

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COMBINATORIAL LIBRARY OF ARTIFICIAL RIBONUCLEASES

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ABSTRACT:

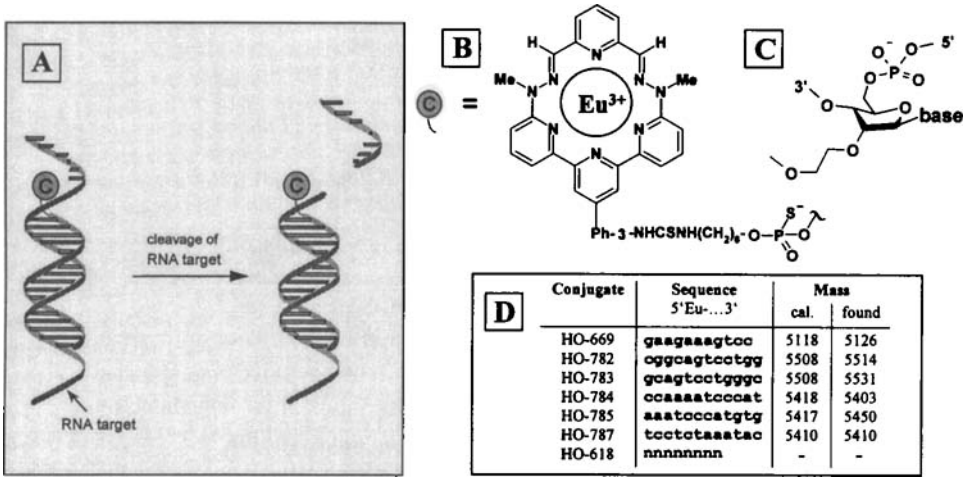
Conjugates of lanthanide complexes and oligonucleotides have been shown to bind and to cleave complementary RNA. Modified oligonucleotide building blocks are required to ensure stability of the oligonucleotide against cellular nucleases. For an efficient cleavage, an accessible site on the structured mRNA is required. For the identification of active oligoribonucleotide-lanthanide complex conjugates, we have used a combinatorial library of conjugate sequences.

Introduction

In 1994, several groups reported the sequence-specific hydrolytic cleavage of RNA using metal complexes covalently linked to deoxy-oligonucleotides, which was also recently reviewed.[1] In our approach, we linked europium complexes to 2'-*O*-methoxyethyl- modified (MOE) oligoribonucleotides suitable for cellular and animal studies (**FIG. 1**). Such artificial nucleases have a wide field of applications as specific RNA cleavage reagents with potential application in cellular assays as well as for pharmaceutical use. One of the principal problems, however, is the identification of conjugates which actively cleave the target RNA. One possible solution to this problem is the use of a library of such conjugates. Treatment of an RNA target with a random mixture of all possible conjugates should reveal those sites which are the most susceptible to cleavage, i.e. hot spots. In this communication we present the use of such 'cleaver libraries' as a means for the rapid identification of potent cleaver conjugates.

Materials and Methods

MOE oligoribonucleotides where synthesized according to the described procedure[2] using an ABI 394 DNA synthesizer. For the synthesis of libraries, the phosphoramidites



D

Conjugate	Sequence 5'Eu-...3'	Mass cal.	Mass found
HO-669	gaagaaagtcc	5118	5126
HO-782	cggcagtcctgg	5508	5514
HO-783	gcagtcctgggc	5508	5531
HO-784	ccaaaatcccat	5418	5403
HO-785	aaatcccatgtg	5417	5450
HO-787	tcctctaaatac	5410	5410
HO-618	nnnnnnnn	-	-

FIG. 1:
A: Artificial ribonuclease. **B:** Formula of the conjugated complex. **C:** Structure of the modified 2'-MOE building block. **D:** Sequence and mass of individual conjugated 2'-MOE oligonucleotides.

were mixed in a ratio, corrected for the difference in the coupling reactivities of the individual amidites. Covalent attachment of the metal complexes to oligoribonucleotides *via* a thiourea linker was carried out using reported methods.[3] Individual cleaver conjugates were characterized by capillary electrophoresis and mass spectrometry (**FIG. 1**). For the cleavage assay the T7-transcribed RNA was labeled with [^{33}P]-γATP and incubated with the cleaver conjugates without pre-heating under non-denaturation condition at 37°C, as in previous studies.[4,5]

Results

Coupling of a complex to the 5'-end of an amino modified 2'-MOE oligonucleotide leads to potent conjugates (**FIG. 1**) which cleave sequence-specifically, short chemically-synthesized (30 nt) RNAs, as well as RNA transcripts up to a length of up to 3000 nt in an *in vitro* assay (unpublished results). Two different paths were followed to identify active cleaver conjugates and were compared, tested against the same mRNA (221 nt part of C-raf mRNA). First, example in **FIG. 2 A** shows a cleaver conjugate (**HO-669**) which was synthesized based upon a published antisense oligonucleotide (ISIS 5132)[6] known to

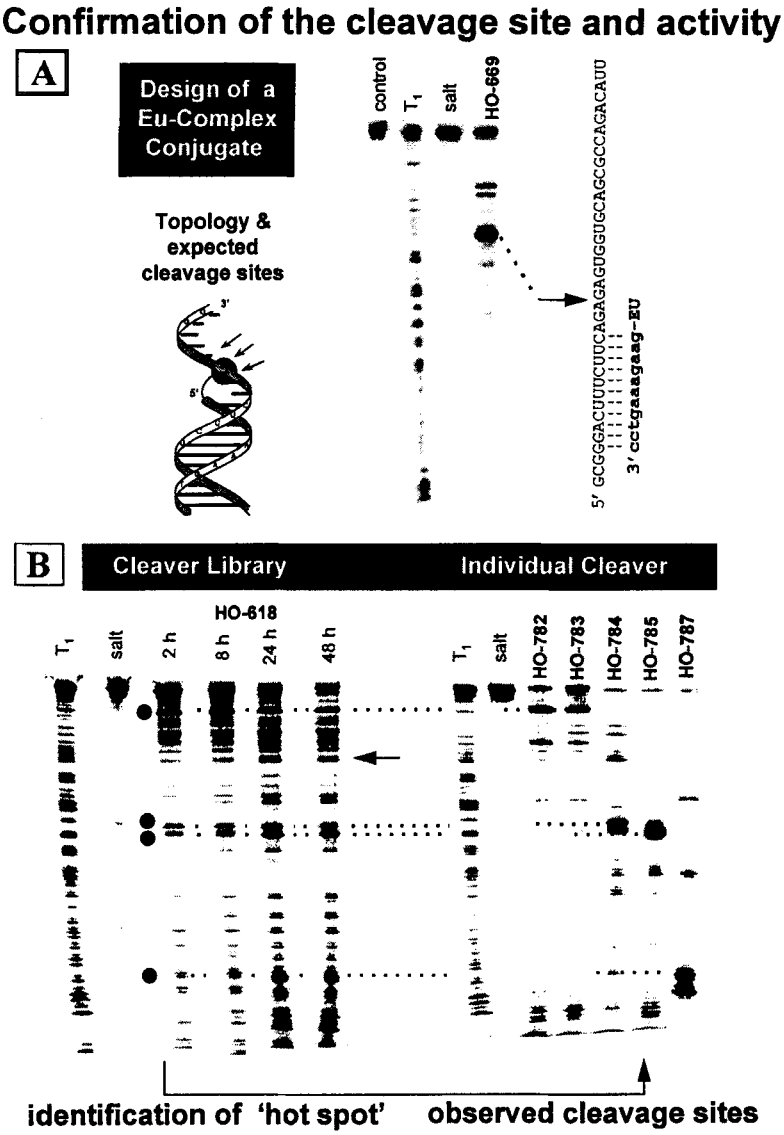


FIG. 2:

Autoradiograms of a 6 % denaturing polyacrylamide gel obtained after treatment of a 221 nt 5'-[^{33}P]-labeled RNA in the presence of 2'-MOE conjugate(s). The incubations for the individual cleaver and the cleaver library were carried out at 37°C in a reaction mixture containing 1 μM conjugate(s) and approximately 10-50 nM RNA in a buffer of 20 mM Na-phosphate pH 7.2, 180 mM KCl, in a total volume of 10 μl. **A:** Left, expected cleavage at a mRNA region known to be accessible to antisense oligonucleotides. Right, the incubation time was 16 h. Small letters are used to designate MOE nucleotide building blocks. The primary cleavage site is indicated by an arrow. **B:** For the library the incubation time varies as indicated and for the individual cleaver it was 16 h. The cleavage site of the cleaver **HO-669** is indicated by an arrow. The hot spots are marked by black bullets.

bind to C-raf mRNA and induce RNase H mediated degradation. Based on previously established rules for linker type and length, and chemically favored cleavage sites on the mRNA, a cleaver conjugate was designed and found to cleave its target with high cleavage activity (**FIG. 2 A**) *in vitro*.

Second, a random library of MOE 8 mer oligoribonucleotide conjugates (**HO-618**, **FIG. 2 B** left side) was tested in parallel. Analysis of the cleavage fragments reveals several hot spots of cleavage (marked with black bullets). Cleavage sites and derived sequences thereof were directly identified using T1 and NaOH sequence ladders (NaOH lane not shown). The corresponding individual conjugates were synthesized as 12 mers and were incubated with the target (**FIG. 2 B**, right side). Three of the conjugates (**HO-784**, **-785** and **-787**) showed considerably higher potency than the other two (**HO-782** and **-783**) (by comparison of remaining RNA starting material), which were of similar potency to **HO-669**.

The experiments described here demonstrate the efficient sequence-specific cleavage of RNA by a random 8 mer library of oligoribonucleotide conjugates. Surprisingly, this cleavage was observed upon treatment with a total concentration of 1 μM of the conjugate library, i.e. with a theoretical concentration of 15 pM for the individual conjugates. This may be an indication of enhanced activity at an optimal cleavage site. Alternatively, it may result from repeated (turn-over) cleavage due to the short length (8 mers) of the conjugates used in the library.

Summary and Conclusion

We have shown that the use of a cleaver library (8 nt length) can provide a method for identifying short MOE antisense oligoribonucleotides (12 mer) conjugates capable of cleaving a given RNA sequence with high efficiency. The identified conjugates cleave their target with equal or higher efficiency than a cleaver conjugate based on a previously identified oligonucleotide sequence with a known accessible target site. Such a cleaver library may serve as a tool for testing the accessibility of binding sites of a given RNA target in solution.

References

[1] Häner, R.; Hall, J. *Antisense Nucleic Acid Drug Dev.*, **1997**, 7, 423-430.
[2] Martin, P. *Helv. Chim. Acta*, **1995**, *78*, 486-504; **1996**, *79*, 1930-1938.

[3] Häner, R.; Hall, J.; Pfützer, A.; Hüsken, D. *Pure & Appl. Chem.*, **1998**, *70*, 111-116.
[4] Hall, J.; Hüsken, D.; Häner, R. *Nucleic Acids Res.*, **1996**, *24*, 3522-3526.
[5] Hall, J.; Hüsken, D.; Häner, R. *Nucleosides & Nucleotides*, **1997**, *16*, 1357-1368.
[6] Leeds, J. M.; Geary, R. S.; Henry, S. P.; Glover, J.; Shanahan, W.; Flitchett, J.; Burckin, T.; Truong, L.; Levin, A. A. *Nucleosides & Nucleotides*, **1997**, *16*, 1689-1693, and references therein.