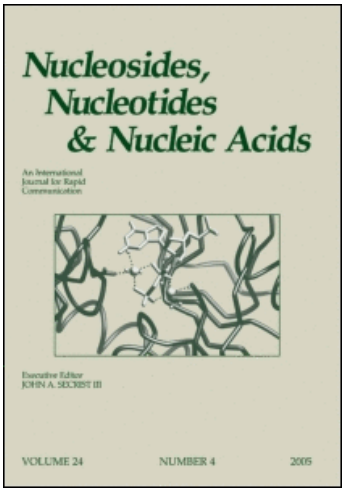

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Oligonucleotide-Peptide Conjjugates for RNA Cleavage

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OLIGONUCLEOTIDE-PEPTIDE CONJUGATES FOR RNA CLEAVAGE

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ABSTRACT Directed cleavage of RNA phosphodiester bonds by peptidyloligodeoxyribonucleotide containing the peptide residues with the alternated hydrophobic and basic amino acids was demonstrated.

Complementary addressed reagents have the widest application for site-directed modification and cleavage of DNA. The cleavage of RNA can be carried out by oxidizing-reducting reagents or by reagents acting as hydrolytic base catalysts. The last ones are the most promising, because they are able to cleave phosphodiester bonds only in RNA without damaging the other biopolymers and DNA.

The main goal of this work was the study of interaction between RNA targets and peptidyloligonucleotide containing the alternated hydrophobic and basic amino acids. As was shown[1] earlier the oligopeptides themselves composed of alternated hydrophobic and basic amino acids are able to cause statistical hydrolysis of phosphodiester bonds of the homological sequences of oligo- and polyribonucleotides. The attachment of such oligopeptides to an oligonucleotide address may lead to the cleavage of RNA in the unique beforehand given site. Since the peptide residue linked to oligonucleotide can influence the hybridization properties of the last one, the thermal denaturation of complexes formed by NA target and peptidyloligonucleotides was examined. All studies were made on the model duplex, where the target strand was DNA analog of the RNA:

5'-dpC A C A C A C A A A A A A C
d T G TG T G T Gp-***Pep***

-***Pep*** = -NH-(*ArgLeu*)$_n$-*Gly*-amide (n=2,3,4)

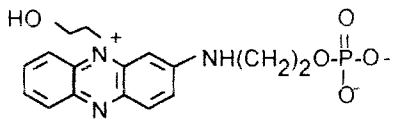
TABLE 1. Melting points of complexes formed by dpCACACACAAAAAAC with the octadeoxyribonucleotide or its oligopeptide conjugates

Peptidyloligonucleotides	T_m,°C
3'-TGTGTGTGp	43,6
3'-TGTGTGTGp-(*ArgLeu*)$_2$-*Gly*-amide	46,0
3'-TGTGTGTGp-(*ArgLeu*)$_3$-*Gly*- amide	47,3
3'-TGTGTGTGp-(*ArgLeu*)$_4$-*Gly*- amide	48,6

The data obtained show that peptide residues do not hinder the formation of the complexes of DNA target with peptidyloligonucleotides. Correlation of duplex stability with length of peptide residue was found.. Each supplementary pair of *ArgLeu* increase T_m of complex by 1.3°C in buffer 0.1 M NaCl, 0.01 M sodium cacodylate (pH 7.4), 1mM EDTA using 1.3 x 10^{-5} M concentration of each oligonucleotide components (TABL.1).

A possibility of the site-directed cleavage of RNA by oligodeoxyribonucleotide-peptide conjugates was studied using as RNA target tetradecaribonucleotide rpN$_{14}$ (the fragment of anticodon loop of tRNA^{Phe} *E.coli*).

X - = HO-; *Phnl*-



The introduction of N-(2-hydroxyethyl)phenazinium residue (*Phn*) to the 3'-phosphate of the hexadeoxyribonucleotide pN$_6$ increases the stability of its complementary complex[2]. It was found that examined peptidyloligonucleotides and their phenazinium derivatives can cleave the RNA target, however the depth and direction of the reaction depend on the number of *Arg* residues (FIG.1).

Practically there are no observed products of hydrolysis of the RNA target by reagent ***Pep***-pN$_6$ (n=3) (FIG.1, line 8). The use of phenazinium derivative ***Pep***-pN$_6$p-*lPhn*

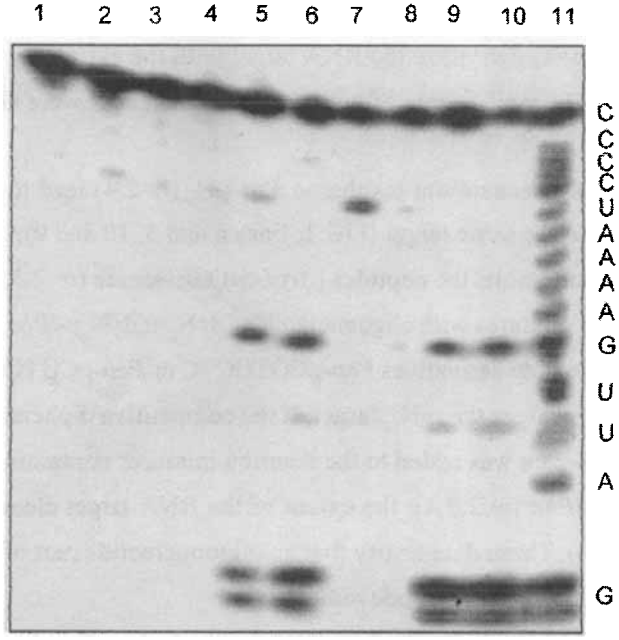


FIG.1. Autoradiogram of the 20% denaturating polyacrylamide gel-electrophoresis of products of hydrolysis of r[^{32}P]GAUUGAAAAUCCCC (**1**) (10^{-7}M) in 0.1 M NaCl, 0.6 mM spermine, 1 mM EDTA, 10 mM tris-HCl, pH 7.5, (48 h, 20°C) by peptidylhexanucleotides (10^{-4} M) and controls:
2 - (*ArgLeu*)$_4$*Gly*-amide;
3 - ***Pep***-pCGTCCTC (n=4);
4 - ***Pep***-pCGTCCTCp-*lPhn* (n=4);
5 - ***Pep***-pTCAATCp-*lPhn* (n=2);
6 - ***Pep***-pTCAATC (n=2);
7 - ***Pep***-pTCAATCp-*lPhn* (n=3);
8 - ***Pep***-pTCAATC (n=3);
9 - ***Pep***-pTCAATCp-*lPhn* (n=4);
10 -***Pep***-pTCAATC (n=4)
11 - OH

(n=3), which forms the more stable complex with rpN$_{14}$, lead to preferable hydrolysis of U10-C11 bond with the yield of about 20% (FIG.1, line 7).

The correlation between hydrolysis and stability which was found for reagent n=3 was different in the case of n=2,4. It was shown that the extent of RNA target hydrolysis decrease when the complex of ***Pep***-pN$_6$ (n=2) with rpN$_{14}$, which is less stable then ***Pep***-

pN_6 (n=4) with rpN_{14}, was used. (FIG.1, lines 6 and 10). Furthermore peptidyloligonucleotide ***Pep***-pN_6 (n=4) hydrolyze the RNA target with the extremely high yield (80%) (FIG.1, line 10). The main points of hydrolysis in both cases were G1-A2 and G5-A6 bonds.

Attachment of phenazinium residue to ***Pep***-pN_6 (n=2,4) lead to decrease of their ability to hydrolyze the same target (FIG.1, lines 6 and 5, 10 and 9).

In the same conditions the peptides $(ArgLeu)_nGly$-amide (n=2,3,4) (FIG.1, line 2, for example), their mixtures with oligonucleotides dpN_6 or dpN_6p-*lPhn*, and non-complementary oligonucleotide derivatives ***Pep***-pCGTCCTC or ***Pep***-pCGTCCTCp-*lPhn* (lines 3 or 4) were not hydrolyze the rpN_{14} target. If the competitive diphenazinium derivative *Phnl*-pCTAACTp-*lPhn* was added to the reaction mixtures containing RNA target and ***Pep***-pCTAACTp-*lPhn* (n=2,3,4), the extent of the RNA target cleavage dramatically decreased (till 5%). These data testify that an oligonucleotide part of such reagents responsible for direct action of peptide moiety.

The proposed reagents don't affect DNA but hydrolyze the phosphodiester bonds of RNA site-specifically. The data obtained show that localization of cleavage site and efficiency of RNA hydrolysis strongly depend on the number of *Arg* residues.

Thus, the oligonucleotide-peptide conjugates are able to cleave the RNA like artificial RNases.

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