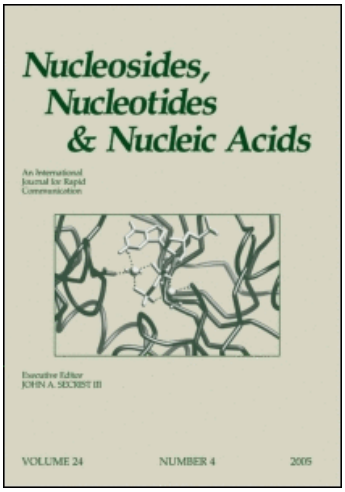

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Catalytic DNA and RNA for Targeting MDR1 mRNA

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ABSTRACT

Design, synthesis and properties of catalytic NAs for targeting MDR1 mRNA are reported.

Key Words: 10–23 DNAzyme; Oligo(2′-O-methylribonucleotide); Effector; Binary ribozyme.

Catalytic nucleic acids capable of cleaving RNA within specific sequences[1] provide powerful tools for inhibition of gene expression at the level of mRNA. The goal of the present study was to design NA enzymes targeted to the mRNA of multi-drug resistance gene MDR1.

Hammerhead ribozyme and DNAzymes 10–23 complementary to three different sites within the 5′-end fragment of MDR1 mRNA, in the translation initiation region and in the coding region were synthesized. ''Inverted'' thymidine was introduced at

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Table 1. Kinetic parameters of RNA cleavage by NA enzymes.

MDR1 mRNA site	NA enzyme	K_m, nM	k_{cat}, min^{-1}	k_{cat}/K_m, $min^{-1}\cdot\mu M^{-1}$
120–137	Dz-120	95,0 ± 6,4	0,021 ± 0,001	0,22
120–137	Dz-120-invT	23,8 ± 7,4	0,025 ± 0,002	1,1
319–333	Dz-319-invT	111,8 ± 25,0	0,091 ± 0,014	0,81
127–145	Dz-127	67,0 ± 26,6	0,60 ± 0,06	8,9
127–145	Dz-127-invT	24,0 ± 1,3	0,24 ± 0,01	10,0
127–145	Rz-127	85,8 ± 11,4	0,21 ± 0,02	2,4

Concentrations of substrates varied from $0.5\bullet10^{-7}$ to $5.0\bullet10^{-7}$M; 50 mM Tris-HC1 (pH 7.5), 10 mM $MgCl_2$, 37°C.

the 3′-end of the synthesized DNAzyme via 3′-3′-phosphodiester linkage in order to enhance its resistance towards serum nucleases. All synthesized NA enzymes effectively cleave the synthetic fragments of MDR1 mRNA in a catalytic manner. The kinetic parameters obtained (Table 1) are in agreement with the published data concerning cleavage of short RNA substrates with similar catalytic DNA and RNA.[2]

We synthesized a binary ribozyme consisted of two partially complementary oligoribonucleotides capable of assembling into hammerhead structure on the RNA target.

In the presence of equimolar or higher concentration of binary ribozyme the target RNA is cleaved as efficiently as in the presence of full-length ribozyme (Fig. 1A). In a catalytic mode, at $1\bullet10^{-8}$ M concentration of both ribozyme strands, the efficiency of cleavage was dramatically reduced, and the limiting extent of cleavage did not exceed 10%. However, suffice it to raise the concentration of only one of the strands to increase the cleavage efficiency by several times (Fig. 1B), thus the limiting extent of cleavage becomes comparable with that of the full-length hammerhead ribozyme.

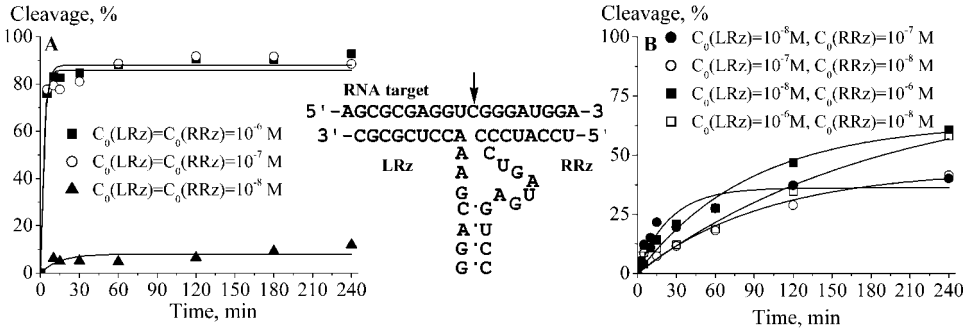

Figure 1. Cleavage of MDR1 mRNA synthetic fragment (nucleotides 127–145) by the binary ribozyme. **A**, equal concentrations of the ribozyme strands; **B**, different concentrations of the strands. RNA concentration was $1.0\cdot10^{-7}$ M; 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 37°C.

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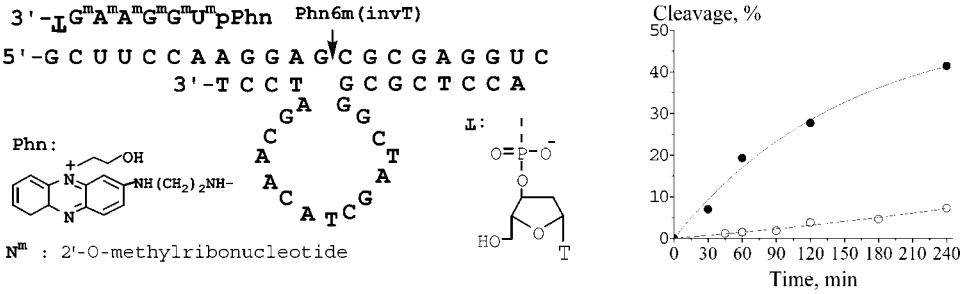

Figure 2. Cleavage of MDR1 mRNA synthetic fragment (nucleotides 117–137) by the shortened DNAzyme 10–23 in the absence (○) and in the presence (●) of effector Phn6m(invT). Concentrations of RNA substrate, DNAzyme and effector were $1.0 \cdot 10^{-7}$ M, $1.0 \cdot 10^{-8}$ M, and $3.2 \cdot 10^{-6}$M, respectively; 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 37°C.

The DNAzyme system containing 10–23 DNAzyme with tetranucleotide 3′-arm was designed (Fig. 2). Oligo(2′-O-methylribonucleotides) (4-, 5- or 6-mer) and their derivatives bearing "inverted" thymidine at the 3′-end and/or the intercalating N-(2-hydroxyethyl)phenazinium (Phn) residue at the 5′-end were used as effectors that bound to the substrate contiguously with the 3′-end of the DNAzyme. The influence of the type, length, and concentration of effectors on the cleavage of RNA by the shortened 10–23 DNAzyme was evaluated.

Increase of the length and concentration of effectors resulted in enhancement of cleavage efficiency. Maximal efficiency of cleavage was observed in the case of the hexamer Phn6m(invT) (Fig. 2). The use of such two-component DNAzymes for the cleavage of the extended structured mRNA would be, probably, even more favorable.

The results obtained indicate that the synthesized NA enzymes can be considered as promising agents for inhibition of MDR1 gene expression.

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