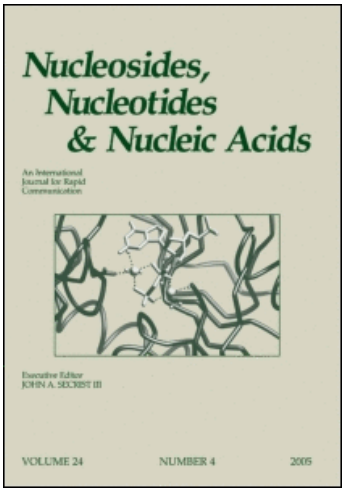

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Two-Component 10–23 DNA Enzymes

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ABSTRACT

A new strategy for engineering of catalytic two-component constructions based on 10–23 DNAzyme was proposed. The using of a combination of shortened DNAzyme with 2′-O-methyl oligomers as effectors significantly increased the catalytic activity of this DNAzyme.

Key Words: Two-component 10–23 DNAzyme; Shortened DNAzyme; Oligo(2′-O-methylribonucleotide) effector; RNA cleavage.

INTRODUCTION

Small catalytic nucleic acids which have the properties of the sequence-specific recognition and site-specific cleavage of RNA provide powerful tools for inhibition of gene expression at the level of mRNA. Recently a new class of catalytic nucleic acids composed entirely of DNA has been obtained by in vitro selection procedure.[1] The 10–23 DNAzyme is the most attractive deoxyribozyme due to its ability to cleave almost any RNA target with high sequence-specificity and efficiency. It consists of a

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15-nucleotide catalytic core surrounded by two substrate recognition domains.[2] It has been reported previously that the use of additional oligodeoxyribonucleotide binding to RNA substrate contiguously with 3′-or 5′-end of DNAzyme noticeably improved its catalytic activity.[3]

To regulate the catalytic activity of DNAzyme we suggested a combination of the 10–23 DNAzyme with oligo(2′-O-methylribonucleotides) on the assumption that they would be more favorable as effectors due to their high affinity to RNA and nuclease resistance.[4–6]

RESULTS AND DISCUSSION

A series of two-component DNAzymes consisting of oligo(2′-O-methylribonucleotide) effectors (6-, 8- and 10-mers) and 10–23 DNAzyme with shortened 3′-binding domain (4 nt) (Fig. 1) was synthesized using phosphoramidite chemistry. To monitor the catalytic efficiency of constructions the 25-mer synthetic fragment of MDR1 mRNA (nucleotides 113–137)[7] was used. First of all we investigated the RNA cleavage by shortened DNAzyme Dz27 without effectors. Under single turnover conditions the extent of RNA cleavage was only 40% after 6 h incubation. In multiple turnover conditions we could not detect any cleavage of RNA. Such loss of DNAzyme Dz27 activity apparently was caused by reduced stability of the Dz27-RNA heteroduplex in consequence of the contraction of its 3′-binding arm.

To test our strategy we investigated the ability of a shortened DNAzyme to cleave the RNA substrate in the presence of an oligo (2′-O-methylribonucleotide) effectors. It

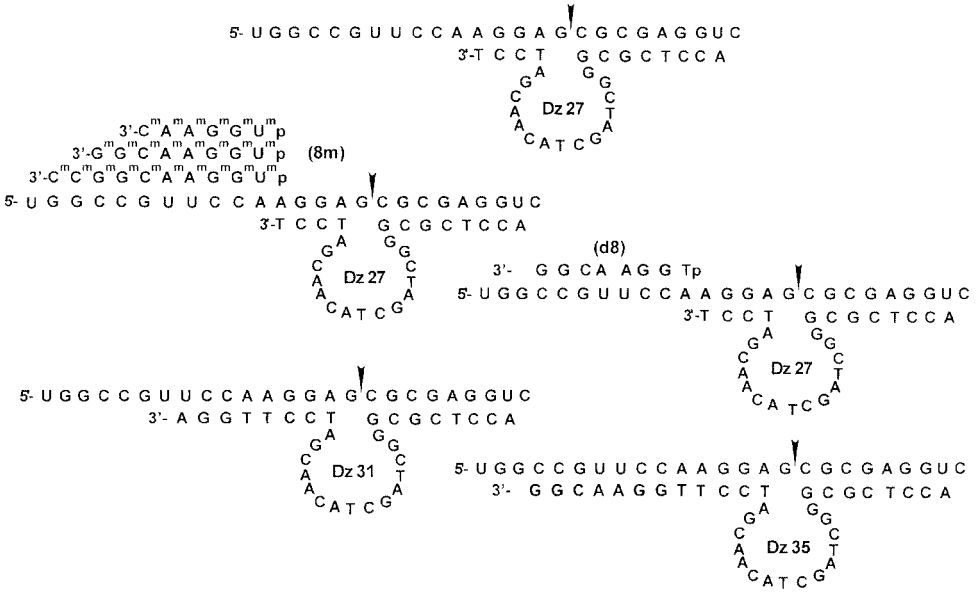

Figure 1. DNAzymes for targeted cleavage of MDR1 mRNA synthetic fragment (nucleotides 113–137).

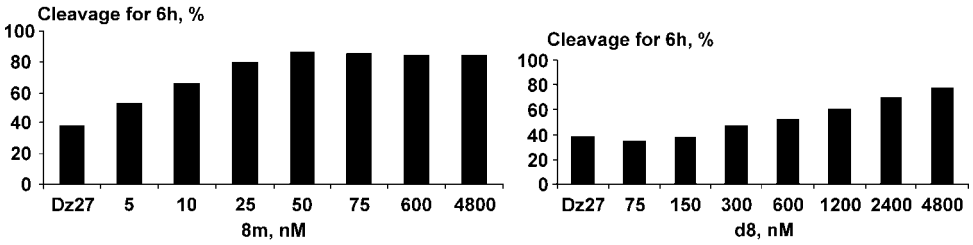

Figure 2. The cleavage of MDR1 mRNA synthetic fragment by DNAzyme Dz27 with 2′-O-methylribo or deoxyribo octamers as effectors (50 mM Tris-HCl. pH 7.5, 10 mM $MgCl_2$. 37°C; DNAzyme 500 nM, substrate 50 nM).

has been displayed (data not shown) that the use of octamer as effector was the most beneficial. At that the octa(2′-O-methylribonucleotide) was noticeably more active as an effector than deoxyribo oligomer of the same length (Fig. 2). The 2′-O-methyl octamer appeared to affect the efficiency of RNA cleavage at 5 nM concentration. The limiting extent of RNA cleavage reached 86% at 50 nM concentration and then remained unchanged. At the same time the octadeoxyribonucleotide appeared to affect the efficiency of RNA cleavage at 300 nM concentration. The extent of RNA cleavage reached 80% after 6 h incubation only at 4800 nM of the deoxyribo effector.

It is known that short oligonucleotides complementary to contiguous sequences on a target RNA show synergistic cooperativity during hybridization,[8] i.e. the helix formed by the first oligonucleotide improves hybridization of the second one by stacking interactions. Consequently the formation of the DNAzyme-RNA helix could be facilitated by the oligonucleotide effector, while the stacking of the effector and DNAzyme could enhance the stability of the DNAzyme-substrate complex. Since 2′-O-methyl oligomers possess higher affinity to RNA, they provide more effective formation of the active complex as compared with deoxy oligomers. The results obtained confirm the fidelity of our strategy to create of two-component DNAzymes.

Finally we performed a comparative study of RNA cleavage by two-component DNAzymes and full-length DNAzymes containing 3′-substrate binding domains

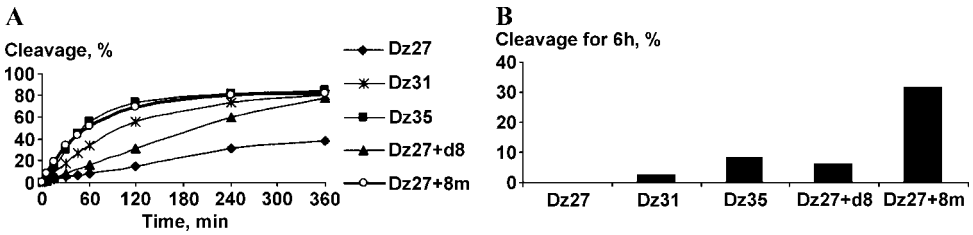

Figure 3. The cleavage of MDR1 mRNA synthetic fragment by two-component and full-length DNAzymes (50 mM Tris-HCl. pH 7.5. 10 mM $MgCl_2$, 37°C). (A) RNA cleavage under single turnover conditions (DNAzyme 500 nM, substrate 50nM, effectors 75 nM); (B) RNA cleavage under multiple turnover conditions (DNAzyme 5 nM, substrate 50 nM, octadeoxyribonucleotide (d8) 4800 nM. octa(2′-O-methylribonucleotide) (8m) 75 nM.

lengthened by 4 or 8 deoxyribonucleotides (Dz31 or Dz35 respectively) (Fig. 3). All reactions were carried out both in single and multiple turnover conditions. Under single turnover conditions the highest cleavage efficiency was observed in the case of binary DNAzyme with octa(2′-O-methylribonucleotide) as effector and also in the case of the lengthened DNAzyme Dz35 probably because these constructions formed the most stable complexes with RNA substrate. However under multiple turnover conditions we observed the distinct differences in the catalytic activity of these DNAzymes. The use of DNAzyme Dz27 with the octa(2′-O-methylribonucleotide) as effector resulted in the highest cleavage extent. In this case the decreased activity of full-length DNAzymes compared with the activity of two-component DNAzyme containing 2′-O-methyl octamer evidently caused by higher stability of complex between cleavage products and full-length DNAzyme Dz35. At that the dissociation of the products is slowed down (more difficult) and therefore the next turnover is inhibited. The detailed study of the kinetic properties of two-component DNAzymes is the subject of our further investigations.

The results obtained confirm the availability of using of oligo(2′-O-methylribonucleotides) as effectors to develop of more powerful DNAzymes for research and potential therapeutic applications.

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REFERENCES

1. Emilsson, G.M.; Breaker, R.R. Deoxyribozymes: new activities and new applications. Cell. Mol. Life Sci. **2002**, *59*, 596–607.
2. Santoro, S.W.; Joyce, G.F. A general purpose RNA-cleaving DNA enzyme. Proc. Natl. Acad. Sci. U. S. A. **1997**, *94*, 4262–4266.
3. Horn, S.; Schwenzer, B. Oligonucleotide facilitators enhance the catalytic activity of RNA-cleaving DNA enzymes. Antisense Nucleic Acid Drug Dev. **1999**, *9*, 465–472.
4. Kuznetsova, M.; Fokina, A.; Lukin, M.; Repkova, M.; Venyaminova, A.; Vlassov, V. Catalytic DNA and RNA for targeting MDR1 mRNA. Nucleosides Nucleotides Nucleic Acids **2003**, *22*, 1521–1523.
5. Freier, S.M.; Altmann, K.H. The ups and downs of nucleic acid duplex stability: structure-stability studies on chemically-modified DNA:RNA duplexes. Nucleic Acids Res. **1997**, *25*, 4429–4443.
6. Novopashina, D.; Kuznetsova, M.; Venyaminova, A. 2′-O-Modified oligoribonucleotides with terminal 3′-3′-internucleotide linkage and their derivatives. Nucleosides Nucleotides Nucleic Acids **2001**, *20*, 903–907.

7. Kostenko, E.V.; Beabealashvilly, R.S.; Vlassov, V.V.; Zenkova, M.A. Secondary structure of the 5′-region of PGY1/MDR1 mRNA. FEBS Lett. **2000**, *475*, 181–186.
8. Maher, L.J., 3rd; Dolnick, B.J. Comparative hybrid arrest by tandem antisense oligodeoxyribonucleotides or oligodeoxyribonucleoside methylphosphonates in a cell-free system. Nucleic Acids Res. **1988**, *16*, 3341–3358.

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