

P008 Binary hammerhead ribozymes as effective tools for specific cleavage of RNA

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Catalytic RNA - ribozymes - combining selective recognition of RNA with site-specific cleavage are the promising tools for mRNA targeting. A large series of binary ribozymes composed of two oligonucleotide strands assembling into a hammerhead structure on RNA target was designed and investigated. Binary ribozymes would take advantage of improved catalytic properties provided by higher rate of products dissociation, and simple synthetic and purification procedures. Using 19-nt MDR1 mRNA fragment as a substrate, it was shown that binary ribozymes are generally more active than parent full-length ones. Kinetic parameters of RNA cleavage were determined. Nuclease resistance of binary ribozymes was enhanced by replacement of all non-conservative ribonucleotides by 2'-OMe or 2'-NH₂ analogs and by the 3'-modification of each strand. Cleavage activity of modified binary ribozymes was similar to that of full-length ribozymes. When 190-nt 5'-terminal fragment of MDR1 mRNA with pronounced secondary structure was used as a substrate, all binary ribozymes retain high catalytic activity, while parent full-length ribozymes displayed significantly reduced cleavage efficiency. Obtained results evidence the advantages of newly designed binary ribozymes and allow considering them as effective tools for gene silencing. This work was supported by RFBR (grant No. 02-04-48586), Russian State Program, and grant No 50 SB RAS for Interdisciplinary Investigations.