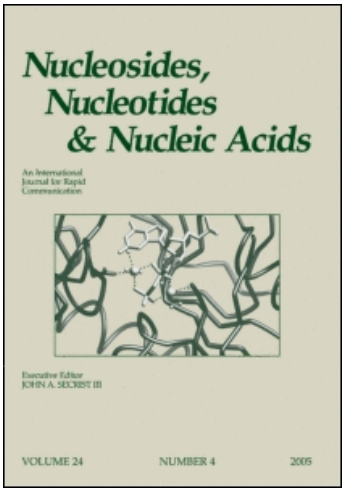

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Construction of a Modified Hairpin Ribozyme for Investigations of the Structure-Function Relationship

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To cite this Article Komatsu, Yasuo , Yamashita, Shigeko and Ohtsuka, Eiko(1999) 'Construction of a Modified Hairpin Ribozyme for Investigations of the Structure-Function Relationship', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1521 — 1522
To link to this Article: DOI: 10.1080/07328319908044775
URL: http://dx.doi.org/10.1080/07328319908044775

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1521–1522 (1999)

CONSTRUCTION OF A MODIFIED HAIRPIN RIBOZYME FOR INVESTIGATIONS OF THE STRUCTURE-FUNCTION RELATIONSHIP

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ABSTRACT: We have constructed a new three domain hairpin ribozyme, in which domain II is connected with domain I' by a helix and a nucleotidic linker to enhance the active bent conformation. The insertion of a nucleotide linker into domain I' increased the cleavage activity more than into domain II. Furthermore, Rev responsive elements (RRE) were introduced in the helix connecting domains. The cleavage activities of the hairpin ribozyme containing the RRE were not affected by the presence of the Rev.

The hairpin ribozyme consists of two domains (domains I and II)[1]. We previously constructed a three domain hairpin ribozyme that exhibits self-trimming activity during transcription (Figure 1a)[2]. Domain I' folds back to domain II by an inter domain interaction, and then cleavage occurs in domain I'. After the cleavage, the released 5'-side fragment can cleave a target RNA. We have constructed a three domain ribozyme in which domain I' is connected with a helix (helix 5) and a nucleotidic linker to enhance the folding for a high self-cleavage activity (Figure 1b). In the ribozyme (TR(m,n)), domain I' should efficiently interact with domain II. Adenylic acid linkers were inserted in domain I' and domain II, to examine the combinations of the numbers of the linker bases (m and n, respectively), that promoted efficient interactions. In these ribozymes, TR(0,5), which is m=0 and n=5, had the highest self-cleavage activity among the four ribozymes (TABLE 1). This result indicates that the linker inserted in domain I' is more important than that in domain II. The self-cleavage activity of TR(0,5) is about five-fold higher than that without helix 5, which was reported previously[2]. It is thought that helix 5 enhanced the folding of the ribozyme.

FIGURE 1

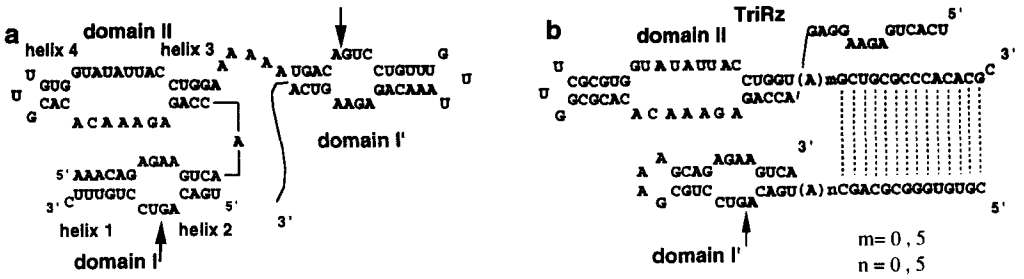

TABLE 1

ribozyme	k_{obs} (min^{-1})
TR(0,0)	0.09
TR(5,0)	0.23
TR(0,5)	0.34
TR(5,5)	0.25

The cleavage activity of the TR(m,n) series ribozymes can not be controlled. Therefore, we constructed ribozymes in which helix 5 was replaced by the RRE sequence[3] to investigate whether the cleavage activity can be controlled by the Rev protein. If Rev increases the self-cleavage activity, then domain I' might be cleaved in cells infected by HIV, and cleavage of the HIV mRNA would occur by the released hairpin ribozyme. The results from in vitro experiments showed that self-cleavage of TR(m,n)-RRE could not be controlled by the Rev protein. These ribozymes exhibited cleavage activity in the absence of Rev. However, the activity was not inhibited by the protein.

The three domain ribozymes that we constructed here confirm the bent conformation of the hairpin ribozyme and exhibit high trimming activity. If helix 5 is replaced by other sequences, the self-cleavage activity may be controlled.

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