Design and properties of trans-acting HDV ribozymes with extended substrate recognition regions

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ABSTRACT
We constructed a new type of trans-acting HDV ribozyme which is based on the antigenomic RNA sequence and has an additional binding site to form an extra stem (P5) of 7 base-pairs introduced in the J1/2 region between P1 and P2 stems. A substrate RNA containing the two binding sequences was specifically cleaved while no selectivity was observed in the case of the wild-type ribozyme with only one binding site. Mutation to produce two mismatch base-pairs in the central part of the P5 stem abolished the specific cleavage.

INTRODUCTION
Human hepatitis delta virus (HDV) has a circular, single-stranded RNA genome of about 1,700 nucleotides. The genome is replicated by a double-rolling-circle mechanism involving linear multimers of both genomic and antigenomic RNAs which have a self-cleaving activity to produce the unit length RNAs (1). The self-cleaving HDV ribozyme system can be modified to a trans-acting ribozyme system which consists of substrate and enzyme components. The trans-acting ribozyme cleaves a substrate RNA in a sequence-specific manner and, therefore, can be used as a tool for abolishing the function of a target RNA which is applicable to gene therapy and analysis of gene functions. For such purpose, the hammerhead ribozyme is mainly used so far. The hammerhead ribozyme is the smallest among the natural ribozymes and its properties, reactions and structure have been studied extensively in vitro and in vivo.

As for the HDV ribozyme which is much larger than the hammerhead ribozyme and has a unique secondary structure, nested double pseudoknot (2,3), only a few such applications are reported. The substrate recognition sequence of both HDV ribozymes is only 7 nucleotide long. The 7 base-pairs for substrate recognition may not be long enough for highly specific targeting when application in vivo is considered. We tried to extend the base-paired region for substrate recognition in trans-acting HDV ribozymes. The trans-acting ribozymes are usually constructed by breaking the RNA chain at or deleting the junction (P1/2) between P1 and P2 stems (Fig. 1b). We introduced an additional stem region (P5) to the trans-acting ribozyme by extending the substrate chain to the 3' direction and the enzyme chain to the 5' direction so that the two extended regions can make base-pairs with each other (Fig. 1c). We prepared mutant ribozymes with an extra substrate binding site of 7 base-pairs and examined the cleavage reactions.

![Diagram]

Figure 1. The scheme for design of trans-acting HDV ribozyme
RESULTS AND DISCUSSION

We designed two HDV ribozyme systems with the extended substrate binding site based on the antigenomic sequence (Fig. 2). The first system consists of two RNA strands, S24 and Rz-E1. The second one consists of three RNA strands, S24 and two RNA components forming Rz-E2. S24 was designed to form P1 stem with 8 base pairs and P5 stem with 7 base pairs and to give a 6-mer cleavage product from its 5'-portion. The sequences for P5 were chosen arbitrarily but the sequences of S24 and Rz-E1 were designed to minimize undesirable intramolecular and intermolecular association. A substrate (S24'), which contains the complementary sequence for P1 stem formation but does not have that for P5 stem, and a ribozyme (Rz-E1W), which contains no extra sequence at the 5'-portion beyond P2 stem, were also designed as control compounds. All RNA oligomers were prepared by in vitro transcription with T7 RNA polymerase. The cleavage reaction was performed at 37°C under single-turnover conditions using 5'-end labeled substrate (< 10 nM) and 1 μM enzyme in 50 mM Tris-HCl buffer (pH 7.0), 10 mM MgCl2.

S24 was cleaved by Rz-E1 but S24' was also cleaved by Rz-E1 with comparable efficiency. A similar result was obtained when Rz-E1W was used as the enzyme although slower cleavage was observed for S24' (Fig. 3). In the case of Rz-E2, S24 was cleaved with a moderate rate. In sharp contrast, S24', which is expected to form only P1 stem, was not cleaved at all (Fig. 3). When one and two mismatches were introduced into the central region of P5 stem, the cleavage activity was greatly reduced by one mismatch and abolished by two mismatches. Thus it is revealed that Rz-E2 can perfectly distinguish the target with two binding sites from that with only one binding site.

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REFERENCES