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Design and switch of catalytic activity with the DNAzyme–RNAzyme combination

Yongjie Sheng, Zhen Zeng, Wei Peng, Dazhi Jiang, Shuang Li, Yanhong Sun, Jin Zhang*

Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, Jilin University, Changchun 130023, PR China

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Abstract **Design and switch of catalytic activity in enzymology remains a subject of intense investigation. Here, we employ a DNAzyme–RNAzyme combination strategy for construction of a 10–23 deoxyribozyme-hammerhead ribozyme combination that targets different sites of the β-lactamase mRNA. The 10–23 deoxyribozyme-hammerhead ribozyme combination gene was cloned into phagemid vector pBlue-scriptIIKS (+). In vitro the single-strand recombinant phagemid vector containing the combination sequence exhibited 10–23 deoxyribozyme activity, and the linear transcript displayed hammerhead ribozyme activity. In bacteria, the 10–23 deoxyribozyme-hammerhead ribozyme combination inhibited the β-lactamase expression and repressed the growth of drug-resistant bacteria. Thus, we created a DNAzyme–RNAzyme combination strategy that provides a useful approach to design and switch of catalytic activities for nucleic acid enzymes.**

Keywords: DNAzyme; RNAzyme; Design; Switch; Catalytic activity

1. Introduction

Design and switch of catalytic activity has made enzymes the subject of the most intense studies of biochemists. Ribozymes and deoxyribozymes are important fields in enzymology, and structural and biochemical research have provided detailed information about the active sites of these enzymes [1–3]. Hammerhead ribozyme is a small RNA motif consisting of three helices that intersect at a conserved core containing GUX triplet (where X is A, C, or U) [4–6]. 10–23 deoxyribozyme, the most active deoxyribozyme, was obtained by in vitro selection [7] and is composed of a conserved catalytic core of 15 nucleotides and two antisense arms of variable length and sequence [8–11]. The hammerhead ribozyme, composed of RNA, was found to be unstable and easily degradable, and additionally lacks a regulation domain, which normally facilitates the formation of complicated spatial structure with moderate rigidity and flexibility. To address the instability of the hammerhead ribozyme, we obtained a circular RNA–DNA enzyme by in vitro selection, which was composed of the hammerhead ribozyme as the active site, as well as backbone DNA with a regulating sequence [12]. Compared to the linear hammerhead ribozyme, the stability of the circular RNA–DNA enzyme was substantially enhanced. Furthering this approach, we substituted the hammerhead ribozyme with the more efficient and more stable 10–23 deoxyribozyme, while the backbone DNA was replaced by the single-stranded replication-competent vector M13mp18 (7.25 kb), thus successfully constructing a novel replicating circular deoxyribozyme, which displayed 10–23 deoxyribozyme activities both in vitro and in bacteria [13,14].

Here, we report the combination of 10–23 deoxyribozyme and hammerhead ribozyme as the catalytic core, which targets the β-lactamase mRNA in initiation and coding regions. The DNAzyme–RNAzyme combination gene was cloned into phagemid vector pBlue-script II KS (+) (2.96 kb). In vitro the single-strand replicas of the recombinant phagemid vector exhibited the 10–23 deoxyribozyme activity and the linear transcript displayed the hammerhead ribozyme activity. In bacteria, both the β-lactamase activity and the bacterial growth of a drug-resistant strain harboring the recombinant phagemid vector containing the DNAzyme–RNAzyme combination gene were inhibited, and these effects were dependent on the catalytic activity. The recombinant phagemid vector containing the DNAzyme–RNAzyme combination sequence had higher RNA-cleaving activity in bacteria than the recombinant phagemid vectors containing either the 10–23 deoxyribozyme or the hammerhead ribozyme. Our experiments indicate that the DNAzyme-RNAzyme combination is capable of replication and transcription in *E. coli* cells and that the activities of the 10–23 deoxyribozyme and the hammerhead ribozyme are intact in vitro and in bacteria. Thus, the switch of catalytic activity could be achieved through the DNAzyme–RNAzyme combination strategy.

2. Materials and methods

2.1. Constructing the recombinant phagemid vectors containing the gene of the 10–23 deoxyribozyme-hammerhead ribozyme combination, the 10–23 deoxyribozyme, the hammerhead ribozyme and their inactive mutants

The complementary DNA fragments (10–23 deoxyribozyme-hammerhead ribozyme, DR; 10–23 deoxyribozyme, D; hammerhead ribozyme, R; inactive mutant 10–23 deoxyribozyme-hammerhead ribozyme, MDR; inactive mutant 10–23 deoxyribozyme, MD and inactive mutant hammerhead ribozyme, MR) (Sangon, Shanghai) for constructing the recombinant phagemid vectors were annealed and ligated (T4 DNA Ligase, TaKaRa, Dalian) with the linear pBlue-script II KS (+) (Merck, German) digested with *Hin*dIII/*Bam*HI (TaKaRa, Dalian). The recombinant phagemid vectors were transformed into

*Corresponding author. Fax: +86 431 88980440.
E-mail address: zhangjin@jlu.edu.cn (J. Zhang).

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E. coli XL1-MRF′ competent cells and spread inoculated onto LB agar plates containing IPTG and X-gal, and the positive clones could be detected for the color varying. Furthermore, the results were tested by sequencing (Sangon, Shanghai).

2.2. Determining the in vitro activities of the replicas and the transcripts of the recombinant vectors

The single-strand circular replicas of the phagemid vectors containing the 10–23 deoxyribozyme-hammerhead ribozyme combination and the 10–23 deoxyribozyme were prepared according to the instruction manual of pBluescript II KS (+) phagemid vector, as well, the linear transcripts of the 10–23 deoxyribozyme-hammerhead ribozyme combination gene and the hammerhead ribozyme were acquired by transcription in vitro (MAXIscript T3 Kit, Ambion). The replicas and the transcripts were purified through agarose gel and PAGE gel, respectively under no RNase contamination conditions. The yields of the replicas and the transcripts were determined from the absorbance of the samples at 260 nm. The multiple-turnover cleavage rates k_{cat} and K_M of the 10–23 deoxyribozymes and the hammerhead ribozymes were determined using different 5′-^{32}P-labelled (T4 polynucleotide kinase, TaKaRa, Dalian) substrates. The substrate of the 10–23 deoxyribozyme was 5′-UGUAUGAGUAUUCAACAUUUUC-3′ (22 nt, TaKaRa, Dalian). The substrate of the hammerhead ribozyme was 5′-UGAGCGUGGGUCUCGCGG-3′ (18 nt, TaKaRa, Dalian).

The experiments of determining the in vitro kinetic parameters of the 10–23 deoxyribozymes were performed at 37 °C in a buffer containing 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$ and 0.01% SDS. The concentration of the non-labelled 10–23 deoxyribozymes was 2 nM, and those of the 5′-^{32}P-labelled substrates from 10 to 240 nM. Aliquots were removed at 5, 15, 30, 60 min, and the reaction was quenched by addition of an equal volume of stop mix (9 M urea, 50 mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue). The substrates and the products were separated on 16% polyacrylamide–7 M urea denaturing gel and visualized by autoradiography. The extent of cleavage was determined from measurements of radioactivity in the substrate and the 5′ products with a Bio-Rad PhosphorImager (Molecular Imager). The kinetic parameters, k_{cat} and K_M, were fitted to the Eadie–Hofstee plot.

Reactions to determine k_{cat} and K_M of the hammerhead ribozymes in 50 mM Tris–HCl, pH 7.5, 100 mM $MgCl_2$ at 37 °C were conducted with hammerhead ribozymes concentration of 25 nM and substrate concentrations ranging from 50 to 500 nM. Aliquots were removed for analysis at different time points (10, 30, 60, and 120 min). Other operations were similar to those of the 10–23 deoxyribozymes described above.

2.3. The activity of the 10–23 deoxyribozyme-hammerhead ribozyme combination in bacteria

Either the plain pBlue-script II KS (+) vector or the recombinant phagemid vectors containing the gene of the 10–23 deoxyribozyme-hammerhead ribozyme combination, the 10–23 deoxyribozyme, the hammerhead ribozyme and their mutants, were electroporated (Micro-Pulser 165–2100, Bio-Rad) into Ampicillin-resistant strain TEM1 (minimum inhibitory concentration, 256 mg/l, obtained from the Bacteria Department of Beijing Hospital). Modified liquid SOC media, in which the concentrations of Amp and the Mg^{2+} were changed to 150 μg/ml and 10 mM, respectively, were used. In order to ensure phase synchronization, similar transformation efficiencies of about $3–5 \times 10^7$ plaques/μg the 10–23 deoxyribozyme-hammerhead ribozyme combination, the 10–23 deoxyribozyme, the hammerhead ribozyme and their mutants were obtained for all transformation experiments. The growth of the bacteria could be determined from the spectrophotometric absorption of cell culture suspensions at 600 nm. The growth inhibition rates were represented by (OD_{ctrl}-OD_{CDR} or$_{CD}$ or$_{CR}$)/OD_{ctrl} (in this formula, ctrl, C_{DR}, C_D and C_R represented the different groups that the drug-resistant strain were electroporated with unrecombinant and recombinant phagemid vectors, respectively). At the same time, the β-lactamase activities of the transformed bacteria were measured according to the iodometry method respectively [15]. The operations were similar with that in Chen's paper [13]. The β-lactamase activity was defined as $\Delta A_{490} = A_a - A_s + A_b - A_c$. A_s represented the absorbance of the sample at 490 nm. A_a, A_b and A_c was the absorbance of the three control experiments respectively, (a) the phosphate buffer (1.25 ml) plus the Amp solution (0.25 ml), (b) the sample cell extract (20 μl) plus the phosphate buffer (1.5 ml) without Amp, and (c) phosphate buffer alone (1.5 ml).

3. Results

3.1. Designing and constructing the 10–23 deoxyribozyme-hammerhead ribozyme combination

The design and schematic construction for the 10–23 deoxyribozyme-hammerhead ribozyme (DR) combination is shown in Fig. 1 and Table 1. We selected two efficient cleavage sites (AU and GUC) within the translation initiation site and the relative conserved sequence of the β-lactamase mRNA as target for the 10–23 deoxyribozyme (D)-hammerhead ribozyme (R) combination [16–22]. Factoring in length of the antisense arms, binding affinity, and the separation of the substrate from the catalytic site, we designed 12/9 nt for the 10–23 deoxyribozyme as well as 6/11 nt for the hammerhead ribozyme in the 10–23 deoxyribozyme-hammerhead ribozyme combination [23,24]. We cloned the designed DR gene into the phagemid vector pBlue-script II KS (+). We simultaneously generated a panel of experimental constructs by cloning wild-type D alone, wild-type R alone, inactive DR mutant (MDR), inactive D mutant (MD) and inactive R mutant (MR) separately into phagemid vectors. Through transforming (*E. coli* XL1-MRF′) and subsequent selection, we acquired the recombinant phagemid vectors, and designated the engineered phagemids containing DR, D, R and their inactive mutants as C_{DR}, C_D, C_R, C_{MDR}, C_{MD}, and C_{MR}, respectively (see Table 1).

3.2. Determining the in vitro activities of the 10–23 deoxyribozymes and the hammerhead ribozymes

We obtained the single-strand C_{DR} and C_D from phagemid preparations of the recombinant vectors. The uncloned sense-strand DNA of the linear 10–23 deoxyribozyme (L_D) was used for the control. We then analyzed the in vitro 10–23 deoxyribozyme activities of C_{DR} and C_D using synthetic RNA substrate. Furthermore, we measured the kinetic parameters of the 10–23 deoxyribozyme for C_{DR}, C_D and L_D (Table 2). The catalytic efficiencies (k_{cat}/K_M) for C_{DR} and C_D were 1.17×10^7 and 1.13×10^7 M^{-1} min^{-1}, respectively, and compared to C_{DR} and C_D activities, the values of L_D were approximately 3-fold greater (3.27×10^7 M^{-1} min^{-1}).

We next analyzed the hammerhead ribozyme activities of the linear transcripts from the recombinant phagemid vectors containing the 10–23 deoxyribozyme-hammerhead ribozyme combination (L_{DR}) and the hammerhead ribozyme (L_R). The kinetic parameters of L_{DR} and L_R were determined using synthetic RNA substrate under the same conditions. As Table 2 showed, the catalytic efficiency of L_R (1.55×10^5 M^{-1} min^{-1}) was higher than that of L_{DR} (4.28×10^4 M^{-1} min^{-1}).

To determine the substrate specificity of the 10–23 deoxyribozyme-hammerhead ribozyme combination, the RNA-cleaving activity of C_{MD} towards the 10–23 deoxyribozyme substrate and C_{MR} activity towards the hammerhead ribozyme substrate were both tested. Neither C_{MD} nor C_{MR} exhibited in vitro activity, indicating that the 10–23 deoxyribozyme-hammerhead ribozyme combination possesses high in vitro substrate specificity.

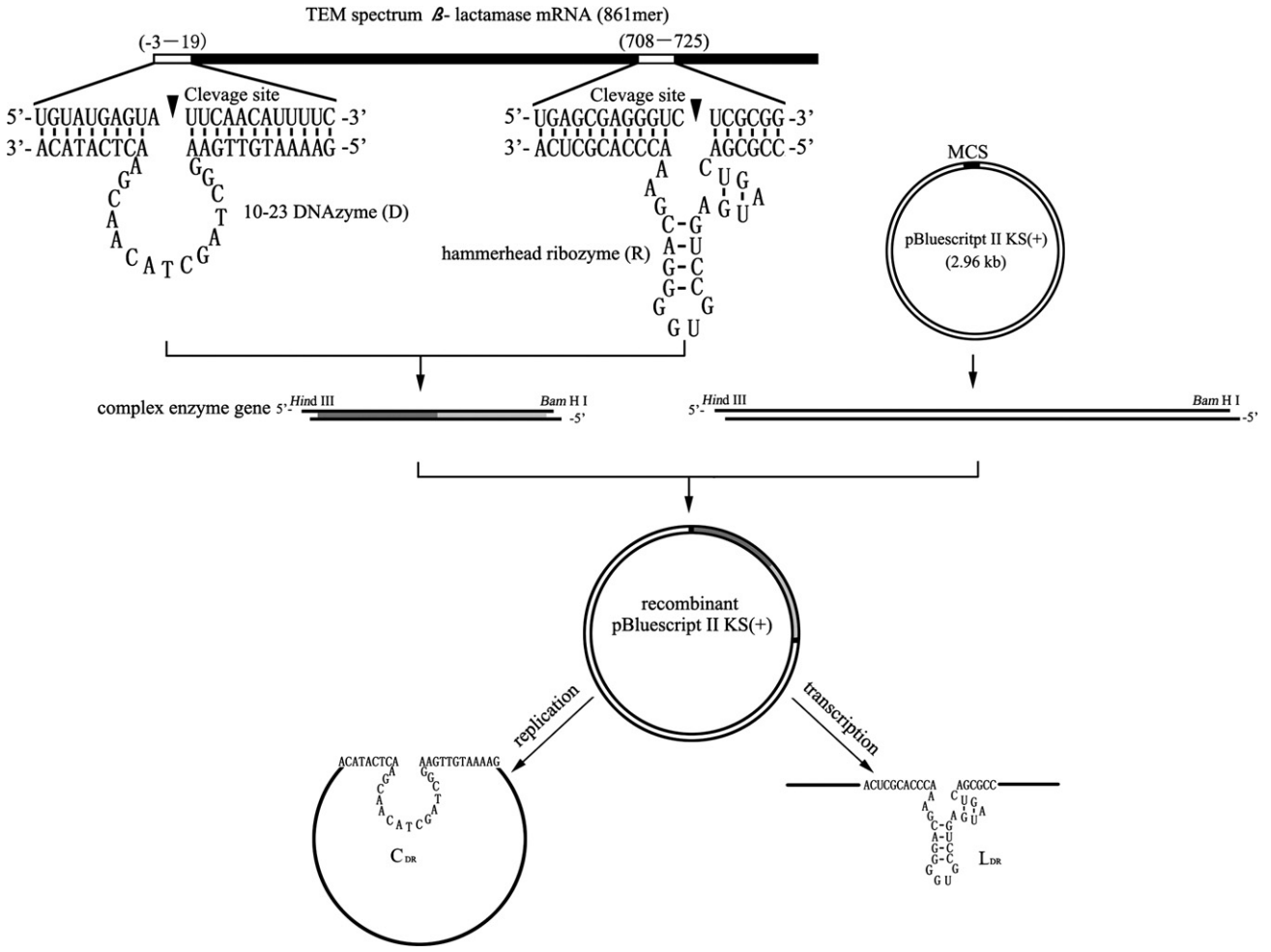

Fig. 1. The design and schematic construction for the 10–23 deoxyribozyme-hammerhead ribozyme (DR) combination. Step 1. The 10–23 deoxyribozyme (D)-hammerhead ribozyme (R) combination was designed to cleave the TEM spectrum β-lactamase mRNA at the indicated sites in the initiation (7–8 for the 10–23 deoxyribozyme) and coding regions (717–719 for the hammerhead ribozyme). Step 2. The complementary cloning fragments (DR) for construction of circular DNAzyme–RNAzyme combination were annealed and ligated with the linear pBlue-script II KS (+) digested with *Hin*dIII/*Bam*HI. Step 3. In the presence of helper phage VCSM13, we acquired the single-strand recombinant vector, which displayed the 10–23 deoxyribozyme activity. Step 4. Lined with *Sac*I, in vitro the transcript (with T3 RNA polymerase) of the DNAzyme–RNAzyme combination gene showed the hammerhead ribozyme activity.

3.3. The dependence of 10–23 deoxyribozyme and hammerhead ribozyme activities on divalent metal ions in vitro

Divalent metal ions are well known and essential cofactors for deoxyribozymes and ribozymes, and play an important role in structure stabilization as well as catalytic activity [25,26]. To determine the dependency of C_{DR} and L_{DR} activity on ion cofactors, several common divalent metal ions were selected and cleavage reactions were performed at pH 7.5 and 37 °C. The results (Fig. 2A and B) revealed that both C_{DR} and L_{DR} activity exhibited metal ion dependency. C_{DR} and L_{DR} showed high activity from 1 mM Mg^{2+} and does not change much until over 50 mM. Neither C_{DR} nor L_{DR} showed activity in the absence of divalent metal ions. Among all the divalent metal ions selected, Mn^{2+}, Mg^{2+} and Ca^{2+} remarkably stimulated the activity of both C_{DR} and L_{DR}. Mn^{2+} and Mg^{2+} were the most efficient activators, while, in contrast, Zn^{2+} and Ba^{2+} had no effect on the activities of either C_{DR} or L_{DR}. Since Mg^{2+} is required for optimal activity, we further tested the dependence of rate of cleavage activity of C_{DR} and L_{DR} in respect to varying concentrations of Mg^{2+} (Fig. 2C and D). The results indicated that, within the range of concentrations analyzed, the activities of both C_{DR} and L_{DR} increased with increasing concentrations of Mg^{2+} as cofactor.

3.4. The activity of the 10–23 deoxyribozyme-hammerhead ribozyme combination in bacteria

The 10–23 deoxyribozyme-hammerhead ribozyme combination could target β-lactamase mRNA, thus acting as β-lactamase inhibitor [27]. This predicts that Amp^r bacteria harboring the 10–23 deoxyribozyme-hammerhead ribozyme combination will lose ampicillin resistance by the inhibition of the β-lactamase expression. To evaluate the RNA-cleaving activity of the 10–23 deoxyribozyme-hammerhead ribozyme combination in bacteria, we transfected Amp^r bacteria TEM-1 by electroporation with the empty pBlue-script II KS (+) vector (ctrl), C_{DR}, C_D, C_R, or their mutants. The growth curves of the corresponding Amp^r bacteria as well as the β-lactamase activities were determined. The ctrl, C_{DR}, C_D and C_R groups with and without ampicillin were analyzed simultaneously, and C_{DR}, C_D and C_R had no detectable effect on TEM-1

Table 1
Cloning DNA fragments

Enzymes and mutants	Cloning DNA fragments
C_{DR}	+(P)5'-AGCTT*CCGCGA**CTGATGAGTCCGTGGGGACGAA***ACCCACGCTCA –3'-AGGCGCTGACTACTCAGGCACCCCTGCTTTGGGTGCGAGT GAAAATGTTGAA**GGCTAGCTACAACGA**ACTCATACAG-3' CTTTTACAACTTCCGATCGATGTTGCTTGAGTATGTCCTAG-5'(P)
C_D	+ (P)5'-AGCTTGAAAATGTTGAA**GGCTAGCTACAACGA**ACTCATACAG -3' –3'- ACTTTTACAACTTCCGATCGATGTTGCTTGAGTATGTCCTAG-5'(P)
C_R	+ (P)5'-AGCTT*CCGCGA**CTGATGAGTCCGTGGGGACGAA***ACCCACGCTCAG-3' –3'- AGGCGCTGACTACTCAGGCACCCCTGCTTTGGGTGCGAGTCCTAG-5'(P)
C_{MDR}	+(P)5'-AGCTT*CCGCGA**CTGATGAGTCCGTGGGGACaAA***ACCCACGCTCA –3'-AGGCGCTGACTACTCAGGCACCCCTG**t**TTTGGGTGCGAGT GAAAATGTTGAA**GGaTAGCTACAACGA**ACTCATACAG-3' CTTTTACAACTTCC**t**ATCGATGTTGCTTGAGTATGTCCTAG-5'(P)
C_{MD}	+ (P)5'-AGCTTGAAAATGTTGAA**GGaTAGCTACAACGA**ACTCATACAG -3' –3'-ACTTTTACAACTTCC**t**ATCGATGTTGCTTGAGTATGTCCTAG-5'(P)
C_{MR}	+ (P)5'-AGCTT*CCGCGA**CTGATGAGTCCGTGGGGACaAA***ACCCACGCTCAG-3' –3'- AGGCGCTGACTACTCAGGCACCCCTG**t**TTTGGGTGCGAGTCCTAG-5'(P)

The DNA fragments for constructing C_{DR}, C_D, C_R and their inactive mutants. The catalytic core of deoxyribozyme or ribozyme is boxed and antisense arms are underlined. The choice of mutant base for the 10–23 DNAzyme is same with our previous results [13]. According to the results of Haseloff [21], the mutant base for the hammerhead ribozyme was designed. The conservation nucleotide mutation of deoxyribozyme or ribozyme are shown as bold lowercase letters, C → a for deoxyribozyme, G → a for ribozyme. As well the shadowed letters at both ends of the fragments represent the restriction site of *Hin*dIII/*Bam*HI.

Table 2
The kinetic parameters of the deoxyribozymes and the ribozymes

Enzymes	k_{cat} (min^{-1})	K_M (nM)	k_{cat}/K_M (M^{-1} min^{-1})
C_{DR}	0.42 ± 0.06	35.76 ± 7.32	1.17×10^7
C_D	0.48 ± 0.06	42.47 ± 7.89	1.13×10^7
L_D	0.89 ± 0.05	27.21 ± 2.21	3.27×10^7
L_{DR}	0.12 ± 0.02	2800 ± 297	4.28×10^4
L_R	0.21 ± 0.02	1360 ± 120	1.55×10^5

The data represent means ± S.D. ($n = 3$).

bacteria growing in the absence of ampicillin. The bacteria also expressed a much lower β-lactamase activity. The results proved that C_{DR}, C_D or C_R groups with ampicillin inhibit the bacteria growth by cleaving the β-lactamase mRNA. As the data in Fig. 3A shows, in the presence of ampicillin, C_{DR} inhibited the growth of drug-resistant strains more efficiently than C_D or C_R. At 10 h when bacteria were in logarithmic growth phase, we observed an OD_{600} difference ($\Delta OD = OD_{ctrl} - OD_{CDR}$) of 0.64 with C_{DR}, 0.30 with C_D, 0.32 with C_R. The growth inhibition rate of C_{DR}, defined as $(OD_{ctrl} - OD_{CDR})/OD_{ctrl}$, was approximately 66%. Fig. 3B showed that at 10 h, the corresponding β-lactamase activity in C_{DR} treated TEM-1 cells ($\Delta A_{490} = 0.18$) were approximately 68% less than levels observed in cells with empty control vector ($\Delta A_{490} = 0.56$). In addition, the three inactive mutants (C_{MDR}, C_{MD}, and C_{MR}) neither inhibited cell growth nor reduced β-lactamase activities, indicating that the growth inhibition was induced by the RNA-cleaving activities of the 10–23 deoxyribozyme-hammerhead ribozyme combination rather than the suppressing of antisense DNA or DNA transfection. Together these results suggest that catalytic activities of C_{DR}, C_D or C_R transfected into drug-resistant bacteria results in the suppression of β-lactamase, likely by β-lactamase mRNA cleavage.

4. Discussion

Cloning the 10–23 deoxyribozyme-hammerhead ribozyme combination gene into pBlue-script II KS (+), the recombinant vectors was constructed and we achieved the switch of catalytic activity through DNAzyme–RNAzyme combination strategy. The 10–23 deoxyribozyme and hammerhead ribozyme activities were confirmed, suggesting the 10–23 deoxyribozyme-hammerhead ribozyme combination gene indeed propagated with the phagemid vector.

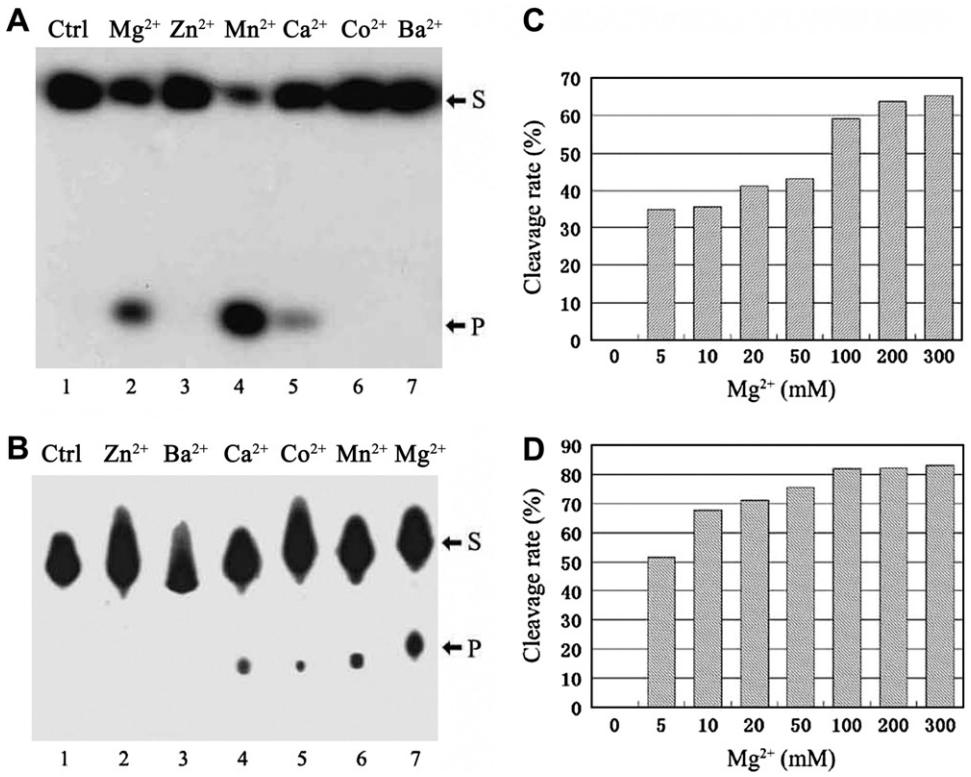

Fig. 2. Dependence of the in vitro activities of C_{DR} and L_{DR} on divalent metal ions. (A) Dependence of the in vitro activity of C_{DR} on divalent metal ions. (B) Dependence of the in vitro activity of L_{DR} on divalent metal ions. (C) Dependence of cleavage rate for C_{DR} on the concentration of Mg^{2+}. (D) Dependence of cleavage rate for L_{DR} on the concentration of Mg^{2+}. Ctrl, substrate without divalent metal ion; S, substrate; P, the products catalyzed with C_{DR} or L_{DR}. Here, 0 concentration of cation indicated no any divalent metal ions were added into the reaction system.

In vitro the deoxyribozyme catalytic efficiencies (k_{cat}/K_M) of both C_{DR} and C_D were lower than that of L_D, which suggested that the active core of C_{DR} and C_D may be conformationally masked, or trapped, by the super coil of the pBlue-script II KS (+) vector [28]. Variations in the catalytic efficiency of C_{DR} in comparison to C_D, were not detectable, implying that the added hammerhead ribozyme gene had little effect on the catalytic efficiency of C_{DR}. L_{DR} contains both the 10–23 deoxyribozyme sequence and the hammerhead ribozyme sequence, thus the extra secondary structure from these sequences is a likely explanation for decreased catalytic efficiency of L_{DR} compared to L_R. Divalent metal ions are important in promoting binding of deoxyribozymes or ribozymes with their substrates and subsequent catalysis. In addition to 10–23 deoxyribozyme and the hammerhead ribozyme binding with divalent metal ions, the vector sequences of C_{DR} and the transcribed deoxyribozyme of L_{DR} could also potentially bind metal ions. This is a possible explanation to why C_{DR} and L_{DR} showed high activity from 1 mM Mg^{2+} and does not change much until over 50 mM, which is different from the linear 10–23 deoxyribozyme or ribozyme alone. In addition, the pBlue-script II KS (+) vector binds metal ions which alter the flexibility and the rigidity of the vector, and accordingly affects to what extent the 10–23 deoxyribozyme-hammerhead ribozyme combination is exposed or masked within the super coil of vectors [29]. Thus, any effect of increased Mg^{2+} concentration on substrate cleavage rate would be weak. The designed RNA substrates were cleaved by C_{DR} and L_{DR}, and the catalytic efficiencies of C_{DR} and L_{DR} were compared to L_D and L_R, as well as the dependence of activities on divalent metal ions in vitro were analyzed. Together these results led to the conclusion that C_{DR} and L_{DR} may adopt similar mechanisms as the10–23 deoxyribozyme and hammerhead ribozyme. Although a few differences distinguish C_{DR} and L_{DR} from the 10–23 deoxyribozyme and hammerhead ribozyme, the catalytic mechanism of C_{DR} and L_{DR} was not affected [3,25,26].

In bacteria, due to the continuous and accumulative effect of the 10–23 deoxyribozyme and the hammerhead ribozyme, C_{DR} had higher RNA-cleaving activity than either C_D or C_R alone. Because the single-stranded circular DNA and the transcript RNA may compete with each other during hybridization to the target β-lactamase mRNA, the cleavage reactions of 10–23 deoxyribozyme and hammerhead ribozyme may interfere with each other. This could explain why the combinatorial effect is not so obvious as we expect. The activity of DNAzyme–RNAzyme combination could be enhanced if the DNAzyme was designed so as not to be transcripted. In the absence of helper phage, pBlue-script II KS (+) exists mainly in its double-strand form in bacteria, thus, contrary to the in vitro data, in these experimental settings C_R showed a higher growth inhibition rate and lower β-lactamase expression than C_D.

Through the switch of catalytic activity, continuous activities at replicating and transcription levels were achieved and the 10–23 deoxyribozyme-hammerhead ribozyme combination exhibits higher RNA-cleaving activity than either the 10–23 deoxyribozyme or the hammerhead ribozyme alone. The strategy described here provides a useful model for design and switch of catalytic activity of enzymes. In addition to 10–23 deoxyribozyme and hammerhead ribozyme analyzed in this

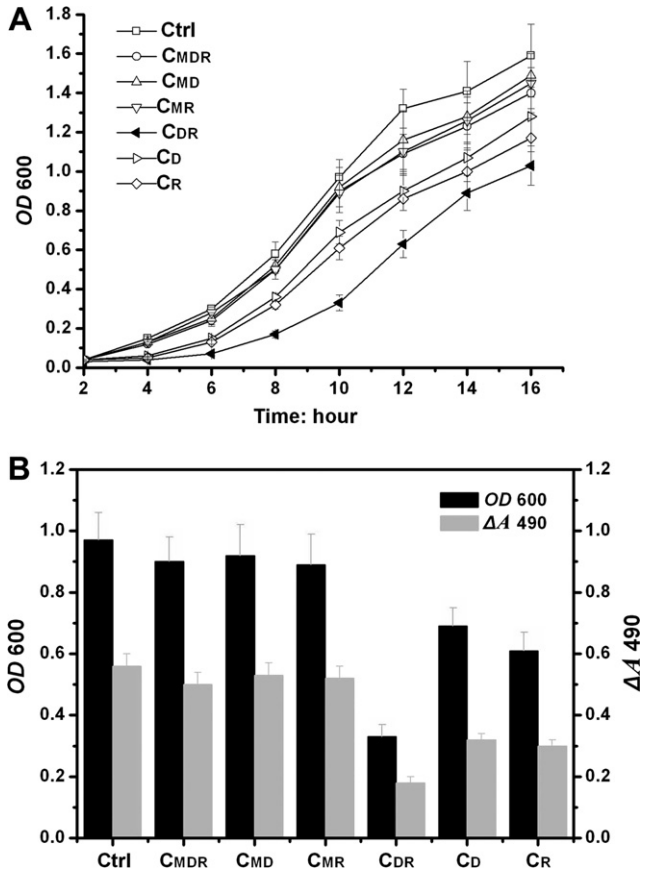

Fig. 3. Activities of C_{DR}, C_D and C_R in the TEM-1 bacteria in the presence of ampicillin. (A) Growth curves of the TEM-1 cells transfected with C_{DR}, C_D, C_R and their inactive mutants, respectively. (B) Growth rates (measured as OD_{600}) and β-lactamase activities (shown as ΔA_{490}) of cultured TEM-1 cells 10 h after the electroporation of the indicated phagemid DNAs. To ensure identical extent of transfection and transcription for different vectors, the same transformation efficiencies were obtained for the transformation experiments. *F*-test was performed to compare the different experiment groups (Ctrl, C_{DR}, C_D and C_R). At 10 h, we obtained an F value of 3.96 ($>F_{0.05}$), and $P < 0.05$ with OD_{600}, and the same value of β-lactamase activity was 4.18($>F_{0.05}$), and $P < 0.05$. All data represented means ± S.D. ($n = 4$).

report, other deoxyribozymes and ribozymes could comprise further novel DNAzyme–RNAzyme combinations. Our model provides a novel approach to design and switch of catalytic activities for nucleic acid enzymes with many practical applications.

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