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Distinct modulation of group I ribozyme activity among stereoisomers of a synthetic pentamine with structural constraints

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ABSTRACT

Among cationic molecules that can modulate ribozyme activities, polyamines act as both activator and inhibitor of ribozyme reactions partly due to their structural flexibility. Restriction of structural flexibility of polyamines may allow them to emphasize particular modulation effects. We examined eight stereoisomers of a synthetic pentamine bearing three cyclopentane rings. In the reaction of a structurally unstable group I ribozyme, three stereoisomers exhibited distinct effects as inhibitor, an additive with a neutral effect, and also as an activator.

1. Introduction

Polyamines are a class of organic molecules that can interact with nucleic acids including functional RNAs [1–4]. Under physiological conditions around pH 7, amine moieties are present predominantly in protonated form (ammonium cation form) due to the pK_a values of their conjugated acids (typically around 10). Organic chemists can design and synthesize polyamines with diverse structures, in which the number and positions of amine moieties can be modulated through the rational design of their backbone skeletons [5–10]. Furthermore, structural design of polyamines enables modulation of their structural rigidity, with which the spatial arrangement of cationic moieties can be finely tuned [6]. One common strategy to restrict the structural flexibility of an organic compound of interest is the incorporation of small-sized cycloalkane structures, such as cyclopentane or cyclohexane, into their main chains [6,8]. These cyclic structures restrict conformational flexibility of the parent skeleton to stabilize one or a few conformations. The molecular structures of major polyamines that occur naturally—such as putrescine, spermidine, and spermine containing two, three, and four amino moieties, respectively—are linear and flexible [1–4]. In living cells, polyamines play important roles in diverse biological processes, some of which involve RNA molecules as their targets [1,3,11–14]. Therefore, polyamines are a promising class of candidate molecules to modulate the structures and properties of functional RNA molecules [15].

Ribozymes, which constitute an important class of functional RNAs, are promising targets of polyamines [3,13,14]. We have investigated natural and synthetic polyamines with linear and flexible skeletons using a structurally unstable group I intron ribozyme (ΔP5 ribozyme, see Fig. 1A) as a model target ribozyme [16–18]. In biochemical analyses of linear polyamines with structural flexibility, increasing the number of amino moieties from two to five caused a gradual decrease in the effective concentrations of polyamines under which they modulate the activity of the ΔP5 ribozyme (Fig. 1B) [18]. These analyses suggested that pentamines may be attractive as a platform for ΔP5 ribozyme modulators [18]. In this study, we exploited the effects of structural rigidity and spatial arrangement of amino moieties using eight stereoisomers of a synthetic pentamine (Fig. S1 in the Supporting Information). The chemical structure of the synthetic polyamine was derived from a flexible and linear pentamine (designated as FLP in this study) shown in Fig. 1C [8]. FLP had five amino moieties connected by three trimethylene units and one tetramethylene unit. FLP also possessed a *p*-methoxyphenyl moiety, which was introduced for its

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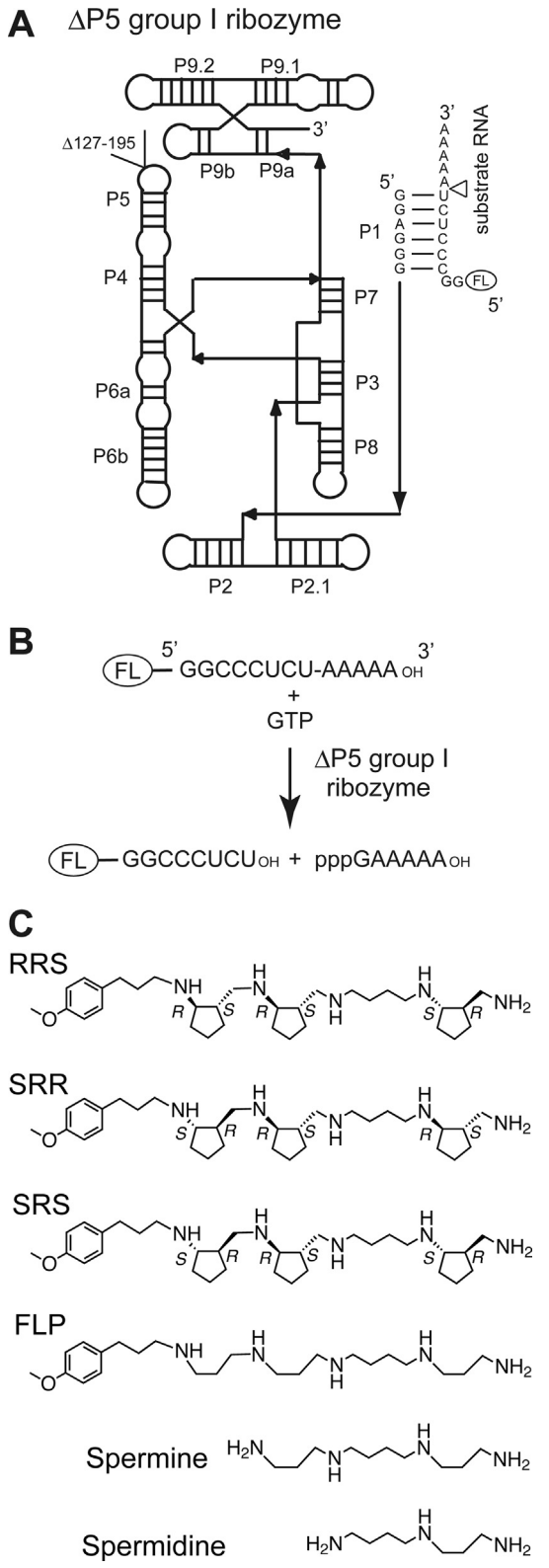

Fig. 1. The ΔP5 ribozyme, substrate cleavage reaction, and synthetic pentamines used in this study

(A) The secondary structure of the ΔP5 ribozyme derived from the *Tetrahymena* group I intron ribozyme by removal of nucleotides 127–195, which constitute the P5abc domain. The ΔP5 ribozyme also lacks the first 21 and last five nucleotides of the *Tetrahymena* group I intron.

(B) The substrate cleavage reaction catalyzed by the ΔP5 ribozyme. A fluorophore (FAM) was linked covalently to the 5′-end of the substrate RNA to visualize its cleavage.

(C) Chemical structures of four synthetic pentamines analyzed in this study. Chemical structures of two naturally occurring polyamines are also shown.

utility in chemical synthesis. Incorporation of three *trans*-cyclopentane rings to the FLP skeleton not only afforded structural rigidity [8] but also generated eight stereoisomers, in which the spatial arrangements of five amino moieties were distinct from one another. Comparative analysis of eight stereoisomers indicated that rigid structures of the constrained pentamines diversified the modulator abilities that may depend on the spatial arrangements of amine moieties.

2. Materials and methods

2.1. Nucleotides and chemicals

Oligonucleotides used as PCR primers were purchased from Fasmac (Tokyo, Japan). The substrate RNA (5′-GGCCCUCUAAAAA-3′), the 5′-end of which was labeled with FAM (carboxyfluorescein) fluorophore, was purchased from JBIOS (Tsukuba, Japan).

2.2. Preparation of synthetic pentamines

Solid-phase chemical syntheses using appropriate building blocks in which the amino groups were protected with Fmoc groups were used to prepare the pentamines used in this study. Synthesis and characterization of the flexible pentamine (FLP) and the RRR isomer of the synthetic pentamine were described previously [8], where FLP and RRR were designated as polyamine 1 and polyamine 2, respectively. Synthesis and characterization of the remaining seven isomers of RRR were also performed in a similar manner.

2.3. RNA preparation

Template DNA for the ΔP5 ribozyme was prepared by PCR as described previously. Plasmid pTZ-ΔP5 encoding the ΔP5 ribozyme was used as the PCR template [19]. The sense PCR primer contained the promoter sequence for T7 RNA polymerase. Transcription reaction with T7 RNA polymerase was performed in the presence of 15 mM Mg^{2+} ions and 4 mM rNTPs for 4.5 h at 37 °C. The DNA template in the reaction mixture was digested by DNase treatment for 30 min. The ribozyme RNA was isolated by electrophoresis on 6% polyacrylamide gels (acrylamide:bisacrylamide 29:1) containing 8 M urea. The concentration of the resulting RNA solution was determined from the absorption at 260 nm.

2.4. Catalytic activity assay

Substrate cleavage reactions (Fig. 1B) were performed at 37 °C in the presence of equimolar amounts of ribozyme RNA and substrate RNA (final concentration: 0.5 μM each). The calculated amounts of RNAs were dissolved in H_2O followed by heating at 80 °C for 5 min and then cooling to 37 °C. To this solution was then added 10 × concentrated buffer, 10 × concentrated H_2O solution of polyamine of interest, and 10 × concentrated GTP (final concentration: 200 μM). The resulting mixture was kept for 30 min at 37 °C. Ribozyme reaction was initiated by adding the substrate RNA. The resulting solution contained 30 mM Tris-HCl (pH 7.5), appropriate amounts of $MgCl_2$ and pentamine, 0.5 μM ribozyme RNA, and 0.5 μM substrate RNA. Aliquots were taken at specific time points and mixed with stop solution containing 80% formaldehyde, 100 mM EDTA, and 0.2% bromophenol blue. The extents of the reactions were analyzed by electrophoresis on 15% polyacrylamide gels (acrylamide:bisacrylamide 29:1) containing 8 M urea. At each time point, the intensities of the substrate and product bands were quantified using Pharos FX fluoroimager (BioRad, Hercules, CA). For data fitting of moderate to fast reactions that completed or nearly

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