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Biogenic triamine and tetraamine activate core catalytic ability of *Tetrahymena* group I ribozyme in the absence of its large activator module

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ABSTRACT

Group I intron ribozymes share common core elements that form a three-dimensional structure responsible for their catalytic activity. This core structure is unstable without assistance from additional factors that stabilize its tertiary structure. We examined biogenic triamine and tetraamine and also their fragments for their abilities to stabilize a structurally unstable group I ribozyme, ΔP5 ribozyme, derived from the *Tetrahymena* group I intron ribozyme by deleting its large activator module. Biogenic triamine (spermidine) and tetraamine (spermine) efficiently activated the ΔP5 ribozyme under conditions where the ribozyme was virtually inactive. These observations suggested that polyamines are promising small molecule modulators to activate and possibly inhibit the core catalytic ability of group I ribozymes.

1. Introduction

RNA molecules with catalytic ability, called RNA enzymes or ribozymes, play crucial roles not only in current biological systems but have also been important in their molecular evolution [1]. Among the several classes of naturally occurring ribozymes, group I intron ribozymes were identified as a unique class of introns that must be eliminated from primary transcripts to produce mature nucleotide sequences. Group I intron RNAs, which exhibit intrinsic self-splicing enzyme-like activity, organize the core machinery to promote this reaction [2]. This self-splicing reaction depends on the folding of group I intron ribozymes into defined and complex three-dimensional (3D) structures [2–4]. Group I intron ribozymes share secondary structural elements (core elements) that fold into a common 3D structure, within which catalysis take place. The periphery of the core elements of group I intron ribozymes is surrounded by structurally variable elements, which are important to wrap and stabilize the core 3D structure. In group I intron ribozymes, the common 3D structure of the core elements is a possible target for small molecules with which their enzyme ability can be directly modulated [5–10].

In this study, we examined biogenic triamine (spermidine) and tetraamine (spermine) and their component diamines as possible activators of the core elements of group I ribozymes. The core structure was derived from the *Tetrahymena* group I ribozyme (Fig. 1A, see also Fig. S1A in the Supporting Information). The *Tetrahymena* group I ribozyme has been studied extensively, and its 3D structure and catalytic mechanism have been elucidated [2–4,11]. The full-length *Tetrahymena* ribozyme is highly active and robust because of a large peripheral extension the P5abc domain (Fig. S1). The P5abc domain serves as a strong activator to stabilize the core elements [12]. Removal of the P5abc domain markedly reduces although does not completely abolish the catalytic ability of the *Tetrahymena* ribozyme [13]. In the resulting variant lacking the P5abc module (ΔP5 ribozyme, see Fig. 1A), the core 3D structure is unstable and the activity can be expressed only in the presence of alternative factors capable of stabilizing the core 3D structure [12–14].

Polyamines are promising candidates as such alternative factors because they have been shown to modulate not only structural

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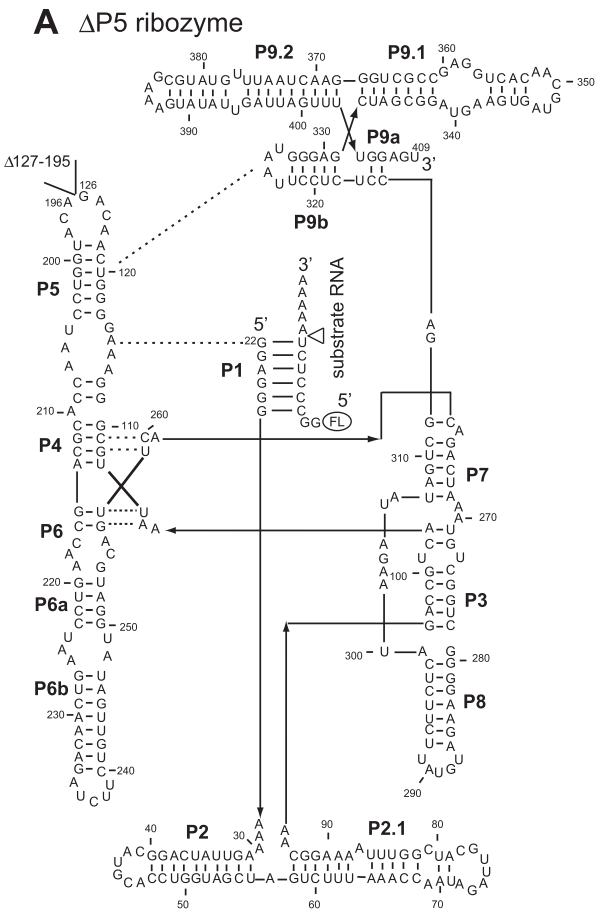

Fig. 1. The group I ribozyme, its reaction, and polyamines used in this study.
(A) The secondary structure of the ΔP5 ribozyme derived from the full-length *Tetrahymena* ribozyme by removal of nucleotides 127–195 that constitute the P5abc extension. The full-length *Tetrahymena* ribozyme (L21 Sca I ribozyme) was also derived from the *Tetrahymena* group I intron by removal of its first 21 and last five nucleotides. (B) The substrate cleavage reaction catalyzed by the ΔP5 ribozyme and the full-length *Tetrahymena* ribozyme. A fluorophore (FAM) was linked covalently to the 5′-end of the substrate RNA molecule to visualize its cleavage.

stability and folding properties of several classes of ribozymes, but also because of their catalytic properties [15]. Koculi et al. analyzed the effects of a series of polyamines on folding of the full-length *Tetrahymena* ribozyme [16,17]. They attempted polyamine-promoted folding of the ribozyme in the absence of Mg^{2+} ions. They then trapped folded RNA structures with 3 mM Mg^{2+} ions in polyacrylamide gels. These data, however, were unlikely to provide direct information regarding the contribution of polyamines to the catalytic activity because the full-length *Tetrahymena* ribozyme is fully active in the presence of 3 mM Mg^{2+} [18]. To address the abilities of polyamines to maintain active ribozyme structures, we used the ΔP5 ribozyme as a structurally unstable ribozyme that is assisted by *trans*-acting factors, such as RNA activators, protein activators, and high concentrations of Mg^{2+} ions.

2. Materials and methods

2.1. Nucleotides and chemicals

The oligonucleotide primers used for PCR were purchased from Fasmac (Tokyo, Japan). The RNA substrate (5′-GGCCCUCUAAAAA-3′), the 5′-end of which was labeled with carboxyfluorescein (FAM), was purchased from JBIOS (Tsukuba, Japan). Each polyamine was purchased from its supplier as its highest grade. 1,3-Propanediamine was purchased from MP Biomedicals Japan (Tokyo, Japan) as a free amine. 1,4-Butanediamine (putrescine) and spermidine were purchased from Nacalai Tesque (Kyoto, Japan) as a dihydrochloride salt and as a trihydrochloride salt, respectively. Spermine was purchased from Sigma-Aldrich Japan (Tokyo, Japan) as a tetrahydrochloride salt. These reagents were used without further purification.

2.2. RNA preparation

Template DNAs for the ΔP5 ribozyme, the full-length *Tetrahymena* ribozyme (called L-21 Sca I ribozyme), and P5abc RNA were prepared by PCR as described previously [19]. The sense PCR primer contained the T7 promoter sequence. Transcription reactions were performed with T7 RNA polymerase, and the desired RNAs were isolated by electrophoresis on 6% polyacrylamide gels (acrylamide:bisacrylamide 29:1) containing 8 M urea. The concentrations of the resulting RNA solutions were determined from the absorption at 260 nm.

2.3. Catalytic activity assay

Substrate cleavage reactions 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 polyamine, 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% formamide, 100 mM EDTA, and 0.2% bromophenol blue. The extents of

(C) Chemical structures of diamines, triamine, and tetraamine used in this study. Under the assay conditions with buffer pH of 7.5, amino moieties in the polyamines were protonated and served as cationic moieties.