Supporting Information:

Phosphoester-transfer mechanism of an RNA-cleaving acidic deoxyribozyme revealed by a new technique involving radioactivity tracking and enzymatic digestion

*Srinivas A. Kandadai, William Chiuman, and Yingfu Li **

Experimental Details

Oligonucleotides and chemical reagents

Standard and modified oligonucleotides were prepared by automated chemical synthesis (HHMI-Keck Biotechnology Resource Laboratory, Yale University; Central Facility, McMaster University) using general phosphoramidite chemistry. DNA oligonucleotides were purified by 10% denaturing (8 M urea) polyacrylamide gel electrophoresis (PAGE). [γ-^{32}P]ATP was purchased from Amersham Pharmacia. T4 DNA ligase, T4 polynucleotide kinase (PNK), calf-intestinal alkaline phosphatase (CIAP) and adenosine 5′-triphosphate (ATP) were purchased from MBI Fermentas. 2′,3′-cyclic nucleotide, 3′-phosphodiesterase (CNPase) was generously provided by Prof. Peter E Braun and Dr. Michel Gravel, McGill University, Canada. All other chemical reagents were purchased from Sigma and used without further purification.

RNA cleavage reaction

The 35-nt sunstrate (**S**, GATGTGTCCGTGCFRQGGTTCGAGGAAGAGATGGC) with ^{32}P-labeled phosphodiester linkage at the cleavage site was constructed as follows: A 50-μL reaction mixture containing 500 pmol of the synthetic DNA oligonuleotide corresponding to the sequence of the last 20 nt of **S** was phosphorylated with 25 μCi of [γ-^{32}P]ATP and PNK (0.1 unit/μL) in 50 mM Tris-HCl (pH 7.6, 25 °C), 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine, 0.1 mM EDTA (pH 8.0, 25 °C) at 37 °C for 30 min, and followed by the addition of non-radioactive ATP to the final concentration of 1 mM and additional 20-min incubation at 37 °C. PNK was inactivated by heating at 90 °C for 2 min. The 5'-phosphorylated DNA was recovered by ethanol precipitation (twice). The DNA was mixed with the synthetic DNA oligonucleotide corresponding to the sequence of the first 15 nt of **S** and a DNA template **T1** (equimolar ratio; its sequence is: GCCATCTCTTCCTCGAACCATAGCACGGACACATC), heated to 90 °C for 30 s, cooled to room temperature, and combined with 0.2 units/μL of T4 DNA ligase and its reaction buffer (40 mM Tris-HCl (pH 7.8, 25 °C), 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP). The resultant solution (300 μL) was incubated for overnight at room temperature. The ligated 35-nt substrate S was purified by 10% denaturing PAGE and resuspended in deionized water. Its concentration was determined spectroscopically using the previously described protocol.[1]

The transesterification reaction was carried out under single-turnover conditions using the above-prepared **S** and the *trans*-acting pH4DZ1 (**E**, its sequence is given in Fig. 1A of the main manuscript). **S** (0.5 μM) and **E** (25 μM) in deionized water were heated at 90 °C for 30 s, cooled to room temperature, prior to the addition of the reaction buffer that contains a final concentration of 50 mM citric acid (pH 3.8, 25 °C), 400 mM NaCl and 10 mM $CdCl_2$. After pre-determined time intervals, a fraction of the reaction solution was taken out, quenched with the stop solution (40 mM EDTA, pH 8.0, 25 °C, 8 M urea, 90 mM Tris, 90 mM boric acid, 10% SDS (w/v), 10% sucrose (w/v), 0.025% xylene cyanol, and 0.025% bromophenol blue) and stored at –20 °C. The reaction mixtures were finally analyzed by 10% denaturing PAGE.

Digestion with protein enzymes

S (0.5 μM) and **E** (25 μM) were heated at 90 °C for 30 s, cooled to room temperature, prior to the addition of the reaction buffer, as described above. After 15 min, the reaction was quenched by the addition of EDTA to a final concentration of 30 mM. The cleaved products were recovered from ethanol precipitation (three times) and the precipitate was re-suspended in 20 μL of deionized water and used for enzymatic probing described below.

Approximately 5 pmol of the cleaved **S** was used in five reactions that involved PNK, CIAP, CNPase, CNPase/PNK, and CNPase/CIAP, respectively. Both PNK and CIAP mediated reactions were carried out at 37 °C for 1 h in a 10-μL reaction mixture containing 10 units of either PNK and CIAP, 10 mM Tris-HCl, pH 7.6, 25 °C, and 10 mM $MgCl_2$. The CNPase mediated hydrolysis of 2′,3′-cyclic phosphate at the end of the 3′-cleavage fragment was conducted at 37 °C for 1 h in a 7 μL mixture containing ~ 5.9 units of CNPase in 50 mM Tris-HCl (pH 7.5 at 25 °C), 50 mM NaCl, 1 mM DTT, and 10% glycerol. The above 7 μL of the CNPase reaction mixture was further incubated with either PNK or CIAP (final reaction volume is 10 μL) at 37 °C for another hour. Following the inactivation of the concerned enzymes at 90 °C for 2 min, each reaction mixture was analyzed on 10% denaturing PAGE. The phosphorimage and fluorimage were both obtained on Typhoon 9200 (Molecular Dynamics).

References:

1. Liu,Z., Mei,S.H., Brennan,J.D. and Li,Y. (2003) Assemblage of signaling DNA enzymes with intriguing metal-ion specificities and pH dependences. *J. Am. Chem. Soc.*, **125**, 7539–7545.