



Supporting Information

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Supplemental Information for

“Structure and Function Converge to Identify a Hydrogen Bond in a Group I Ribozyme Active Site”

Marcello Forconi, Raghuvir N. Sengupta, Mao-Chin Liu, Alan C. Sartorelli, Joseph A. Piccirilli, and Daniel Herschlag

EXPERIMENTAL SECTION

Materials. 3-Deazaguanosine was synthesized by a known procedure.^[1,2] Oligonucleotides were purchased from Dharmacon Inc. (Lafayette, CO) or IDT (Coralville, IA), or synthesized at the University of Chicago. 5'-³²P-end-labeling of the oligonucleotide substrates for kinetic experiments was performed using standard methods.^[3] Oligonucleotides corresponding to nucleotides 260-274 of the ribozyme, containing a 5'-phosphoryl group and a 2'-OH, 2'-OMe, or 2'-H group at position 261 were purified by anion exchange HPLC (0-200 mM NaCl over 3 min, then 200-500 mM NaCl over 30 min in a background of 20 mM Tris, pH 7.4), and desalted by Sep-Pak (Waters, Milford, MA). AUCG and AUCI were purified by anion exchange HPLC (0-200 mM NaCl over 30 min in a background of 20 mM Tris, pH 7.4), and desalted by Sep-Pak (Waters, Milford, MA).

Ribozyme preparation. Wild type *in-vitro* transcribed *Tetrahymena* L-21 *Scal* ribozyme was prepared as described previously.^[3] Variant ribozymes were constructed semi-synthetically using a single-step three-piece ligation.^[4] Constructs corresponding to nucleotides 22-259 and 275-409 of the *Tetrahymena* ribozyme were transcribed using a DNA template produced by PCR truncation of the plasmid-encoded ribozyme sequence, with excess GMP present in the transcription of the 3'-construct (275-409) to yield a 5'-monophosphate. The 5'-construct contained a 3'-flanking hammerhead cassette to ensure homogeneous 3'-ends; the terminal 2',3'-cyclic phosphate was removed after gel purification by treatment with T4 polynucleotide kinase.^[5] The transcripts were ligated to the HPLC-purified synthetic oligonucleotides via a single-step ligation with T4 DNA ligase and a DNA splint to yield full-length ribozyme containing a single mutation at the desired site. To improve yields, 30% glycerol was added to the ligation mixture. Yields were ~15% in purified, fully ligated ribozyme. The ligated ribozymes were ~70% active, but the inactive population did not contribute to oligonucleotide substrate binding and therefore did not affect the cleavage kinetics, which were carried out with ribozyme saturating with respect to the oligonucleotide substrate (see Kinetic methods).

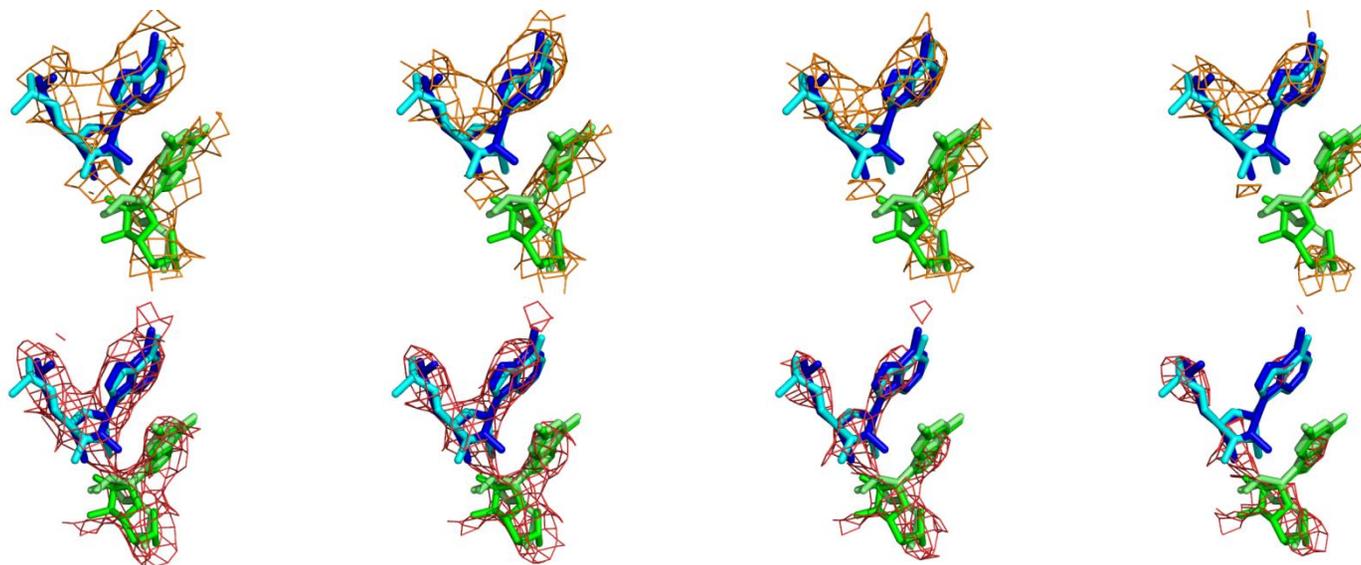
Kinetic methods. All cleavage reactions were single turnover, with ribozyme in excess of radiolabeled oligonucleotide substrate (*S), which was always present in trace quantities (<100 pM). Reactions were carried out at 30 °C in 45 mM NaHEPES/5 mM NaMOPS, pH 8.1, and 50 mM MgCl₂. Reaction mixtures containing 10 mM MgCl₂ and 50 mM NaMOPS, pH 6.9, were pre-incubated at 50 °C for 30 min to renature the ribozyme (present in a

10-fold excess concentration compared to the final reaction conditions). Additional components were added to raise the pH and the MgCl₂ concentration to the desired values, and to dilute the ribozyme concentration to the desired value, and reactions were allowed to equilibrate at 30 °C for 5 minutes before the addition of *S. Reactions were followed by gel electrophoresis and analyzed as described previously.^[6, 7]

$(k_{\text{cat}}/K_M)^{\text{xG}}$ is the second-order rate constants for the reaction $(E \cdot S)_o + \text{xG} \rightarrow \text{products}$, where xG is guanosine (G), 3-deazaguanosine (3dNG), AUCG, or AUCI. In these experiments, we used the oligonucleotide substrate d(CCCUC)rUd(A₅), where 'd' represents a 2'-deoxyribonucleotide and 'r' a 2'-ribonucleotide. This oligonucleotide binds to the wt and modified ribozymes in the open complex [ref. (7) and data not shown]. Values of $(k_{\text{cat}}/K_M)^{\text{G}}$ and $(k_{\text{cat}}/K_M)^{\text{3dNG}}$ were determined with ribozyme saturating with respect to the oligonucleotide substrate (50 nM E) and with subsaturating G or 3dNG (0-25 μM, which is at least four-fold below saturation for all of the ribozymes, data not shown). Values of $(k_{\text{cat}}/K_M)^{\text{AUCG}}$ and $(k_{\text{cat}}/K_M)^{\text{AUCI}}$ were determined with ribozyme saturating with respect to the oligonucleotide substrate (50 nM E) and with subsaturating AUCG (0-0.2 μM) or AUCI (0-50 μM) [these values are at least four-fold below saturation for all of the ribozymes (data not shown)].

		Two	Azo
A261 2'-OH	G 2'-OH	3.1	5.4
A261 2'-OH	G N3	2.6	3.1
A261 2'-OH	G -NH ₂	3.7	2.9

SUPPLEMENTAL TABLE 1. Distances (in Å) of selected residues in the structural models from Twort (Two, PDB ID 1Y0Q)^[8] and *Azoarcus* (Azo, PDB ID 3BO3)^[9] ribozymes crystals.



SUPPLEMENTAL FIGURE 1. Fitting of the electron density maps from the Twort (top, orange mesh) and *Azoarcus* (bottom, red mesh) group I ribozymes to the published structural models of Twort (lighter colors, PDB ID: 1Y0Q, 3.6 Å resolution) and *Azoarcus* (darker colors, PDB ID 3BO3, 3.4 Å resolution) at sigma values = 0.5, 1.0, 1.5, and 2.0 (left to right). Residue A261 (numbering from the *Tetrahymena* ribozyme) is in blue, G is in green. Electron density map for the Twort ribozyme was generated using the CCP4 package^[10] from the structure factors provided by Dr. Barbara Golden. Electron density map from the *Azoarcus* ribozyme was downloaded from the Electron Density Server Database (<http://eds.bmc.uu.se>). Figure was generated with Pymol (DeLano, W.L. The PyMOL Molecular Graphics System (2002) on World Wide Web <http://www.pymol.org>).

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