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Supplemental Information

2′-Fluoro Substituents Can Mimic Native 2′-Hydroxyls within Structured RNA

Marcello Forconi, Jason. P. Schwans, Rishi H. Porecha, Raghuvir N. Sengupta, Joseph A. Piccirilli, and Daniel Herschlag

TABLE S1, related to Table 1. Binding and reactivity of AUCG with closed complexes of different ribozymes, using the -1d,rSA or the -1d,rSA$_5$ substrates.

Numbers in parentheses represent the values relative to the A261OH ribozyme.

	-1d,rSA, pH 6.5		**-1d,rSA$_5$, pH 8.1**	
ribozyme	$(K_d^{AUCG})_c$ (μM)	k_c (min^{-1})	$(K_d^{AUCG})_c$ (μM)	k_c (min^{-1})
A261OH	0.58 ± 0.10 (1.0)	0.076 (1.0)	3.4 (1.0)	1.0 (1.0)
A261F	0.64 ± 0.06 (1.1)	0.017 (0.22)	4.0 (1.2)	0.24 (0.24)
A261H	3.2 ± 0.4 (5.5)	0.0040 (0.053)	31 (9.1)	0.023 (0.023)

TABLE S2, related to Figure 2. Sites of 2′-deoxynucleotide interference in ΔC209 P4-P6 at 0.45 mM $MgCl_2$. Hydrogen bonds are inferred from the ΔC209 P4-P6 crystal structure (PDB ID: 1HR2). Hydrogen bond partners are the residues that engage in a hydrogen bond with the site of 2′-deoxynucleotide interference. The interacting functional group(s) from the hydrogen bond partner are indicated in parentheses. Distances are the average distance between interacting functional groups from the two chains of the asymmetric unit.

2′-OH donor			2′-OH acceptor			2′-OH donor *and* acceptor			2′-OH donor *or* acceptor		
Residue	**Hydrogen bond partner**	**Distance (Å)**	**Residue**	**Hydrogen bond partner**	**Distance (Å)**	**Residue**	**Hydrogen bond partners**	**Distance (Å)**	**Residue**	**Hydrogen bond partner**	**Distance (Å)**
C109	A184 (2′-OH)	2.7	**G201**	G126 (N2)	2.9	**G110**	A183 (2′-OH, N3)	2.7, 2.6	**G126**	U202 (2′-OH)	2.7
C137	A186 (2′-OH)	2.6	**G250**	A152 (N6)	3.1	**A153**	C223 (2′-OH, O2)	2.8, 2.9	**U202**	G126 (2′-OH)	2.7
G150	A152 (N7)	2.9				**U224**	A152 (2′-OH, N3)	2.8, 2.8			
A152	U224 (2′-OH)	2.9									
A183	G110 (2′-OH)	2.7									
G212	A184 (N1)	2.6									
C223	A153 (2′-OH)	2.8									
A248	G250 (O2P)	2.9									

A151 shows weak 2′-H interference, although there is no hydrogen bond inferred from the crystal structure.

FIGURE S1, related to Table 1. AUCG binding to the closed complexes of the A261OH, A261F, and A261H ribozymes. Top: -1d,rSA substrate. Bottom: -1d,rSA$_5$ substrate. Raw data are shown on the left; normalized data on the right. The inset on the right shows the normalized curves at low AUCG concentration. Fitting of the experimental data points to Equation 1 gives the parameters shown in Table S1.

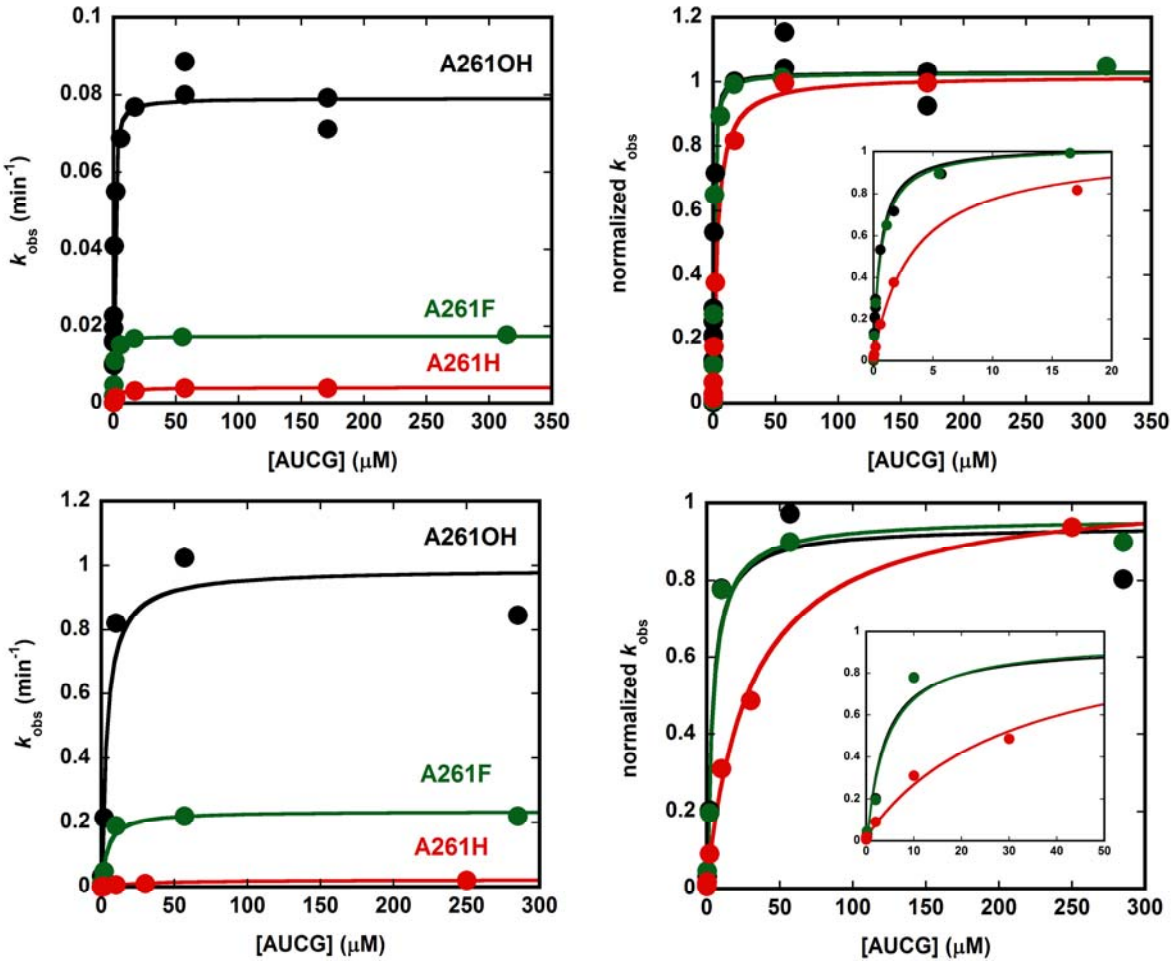
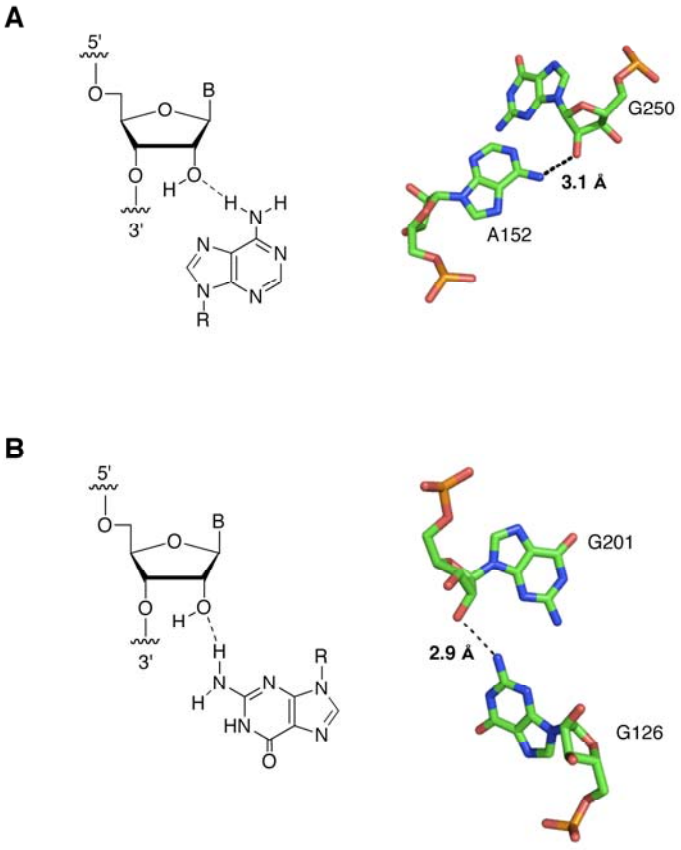


Figure S2, related to Figure 2. 2´-Fluoronucleotides functionally mimic the 2´-hydroxyl group at residues implicated as hydrogen bond acceptors. The 2´-hydroxyl is positioned within hydrogen bond distance from the exocyclic amine group of (**A**) adenosine or (**B**) guanosine. The exocyclic amine group of cytidine is also a potential hydrogen bond donor between an exocyclic amine group and a 2'-hydroxyl group, but the interaction was not observed in the ΔC209 P4-P6 crystal structure and so was not tested in our study (Juneau et al., 2001). Geometric constraints imposed by the exocyclic amine group allowed the amine hydrogen atoms to be modeled into the structure, and indicated an orientation consistent with formation of a hydrogen bond.



SUPPLEMENTAL EXPERIMENTAL PROCEDURES.

Materials. AUCG was synthesized by the PAN Facility (Stanford, CA). Oligonucleotides corresponding to nucleotides 260-274 of the ribozyme, containing a 5′-phosphoryl group and a 2′-OH, 2′-F or 2′-H group at position 261 were synthesized by the PAN Facility (Stanford, CA). Oligonucleotides were purified by reverse-phase HPLC as previously described (Forconi et al., 2010). Wild type and variant ribozymes were constructed semi-synthetically using a single-step three-piece ligation (Moore and Sharp, 1992) with a modified protocol as previously described (Forconi et al., 2010). 2′-Fluoroadenosine-α-thiotriphosphate was purchased from Glen Research. 2′-Fluorocytidine-α-thiotriphosphate, 2′-fluoroguanosine-α-thiotriphosphate, and 2′-fluorouridine-α-thiotriphosphate were synthesized as described below.

General 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). 5′-^{32}P-End-labeling of the oligonucleotide substrates for kinetic experiments was performed by standard methods. The oligonucleotide substrates used in this work are CCCUCdUA (referred to as -1d,rSA), CCCUCdUA$_5$ (referred to as -1d,rSA$_5$), and d(CCCUC)Ud(AAAAA) (referred to as -1r,dSA$_5$). These substrates contain mixed ribose and deoxyribose residues, with deoxyribose residues indicated by a ‘d’, and allow the reactions to be monitored from different E•S complexes (see below). Reactions were carried out at 30 °C with 50 nM ribozyme, 50 mM $MgCl_2$, and 50 mM NaMOPS, pH 6.5 (for reactions of the -1d,rSA substrate) or 45 mM NaHEPES/5 mM NaMOPS, pH 8.1 (for reactions of the -1d,rSA$_5$ and -1r,dSA$_5$ substrates) as previously described (Forconi et al., 2010).

To obtain the kinetic parameters for the A261OH, A261F, and A261H ribozymes, the observed rate constant (k_{obs}) for cleavage of *S was plotted as a function of AUCG concentration and fit to Equation 1 to obtain k_c and K_d^{AUCG}.

$$k_{obs} = \frac{k_c \times [AUCG]}{[AUCG] + K_d^{AUCG}} \quad (1)$$

To determine the kinetic parameters for the open complex, we used the oligonucleotide substrate -1r,dSA$_5$, which favors the open complex and for which the chemical step is rate-limiting (Knitt and Herschlag, 1996). To determine the kinetic parameters for the closed complex we used the -1d,rSA substrate. This substrate contains only one nucleotide after the cleavage site. Previous results have shown that additional nucleotides after the first A interfere with the 5′-part of the nucleophilic AUCG oligonucleotide, and that these steric clashes reduce the binding affinity of AUCG (Russell and Herschlag, 1999). Thus, we decided to use the -1d,rSA substrate to maximize AUCG biding affinity and avoid complications arising from steric clashes. However, reactions with the -1d,rSA$_5$ substrate did not change the relative values of the kinetic and thermodynamic parameters shown in Table 1 (Supporting Table 1). The deoxyribose residue at position -1 ensures that the chemical step is rate-limiting and that the observed $K_{½}^{AUCG}$ equals $(K_d^{AUCG})_c$ (McConnell et al., 1993).

Synthesis and characterization of 2′-fluoronucleoside-α-thiotriphosphates. 2′-Fluorocytidine and 2′-fluorouridine were purchased from ChemGenes and 2′-fluoroguanosine was synthesized as described by Kawaskai et al. (Kawasaki et al., 1993) The nucleosides were converted to the α-

thiotriphosphates and purified as previously described for 2′-mercaptonucleoside-α-thiotriphosphates (Schwans et al., 2003).

^{31}P and ^{19}F NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. ^{31}P chemical shifts are reported relative to a standard of 85% aqueous H_3PO_4. ^{19}F chemical shifts are reported relative to a standard of trifluoroacetic acid.

2′-Fluorocytidine-α-thiotriphosphate. ^{31}P NMR (D_2O, 161 MHz): δ 44.6 m, -10.7 d, -23.0 m; ^{19}F NMR (D_2O, 376 MHz) δ -127. MS (ESI): calculated for $C_9H_{14}FN_3O_{12}P_3S^-$ (M^-): 499.95; found 499.9.

2′-Fluoroguanosine-α-thiotriphosphate. ^{31}P NMR (D_2O, 161 MHz): δ 44.6 dd, -9.7 d, -22.9 m. ^{19}F NMR (D_2O, 376 MHz): δ -126. MS (ESI): calculated for $C_{10}H_{14}FN_5O_{12}P_3S^-$ (M^-): 539.99; found 539.9.

2′-Fluorouridine-α-thiotriphosphate. ^{31}P NMR (D_2O, 161 MHz): δ 44.5 m, -9.7 d, -23.0 m. ^{19}F NMR (D_2O, 376 MHz) δ -126. MS (ESI): calculated for $C_9H_{13}FN_2O_{13}P_3S^-$ (M^-): 500.93; found 500.9.

ΔC209 P4-P6 interference mapping experiments. Interference mapping experiments were conducted as previously described by Schwans et al. and references therein (Schwans et al., 2003).

SUPPLEMENTAL REFERENCES

Knitt, D.S., and Herschlag, D. (1996). pH dependencies of the *Tetrahymena* ribozyme reveal an unconventional origin of an apparent pK_a. Biochemistry 35, 1560-1570.

McConnell, T.S., Cech, T.R., and Herschlag, D. (1993). Guanosine binding to the *Tetrahymena* ribozyme: Thermodynamic coupling with oligonucleotide binding. Proc. Natl. Acad. Sci. U. S. A. 90, 8362-8366.