SUPPORTING INFORMATION

Promiscuous Catalysis by the *Tetrahymena* Group I Ribozyme

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MATERIALS

S_{Me} was purchased from Midlands Certified Reagents, Midlands, TX. S_{ox} was purchased from Integrated DNA Technologies, Coralville, IA. The methoxy groups are introduced to avoid miscleavage and do not affect the reaction.[1]

EXPERIMENTAL

Radiolabeling of substrates. ^{32}P-labeling of oligonucleotides at the 5´-end with [γ-^{32}P] ATP and polynucleotide kinase was carried out essentially as previously described.[2] ^{32}P-labeling of oligonucleotides at the 3´-end was carried out with [α-^{32}P] dATP and terminal nucleotide transferase according to literature procedures,[3] adding a sixth adenosine residue to S_{Me} and S_{ox} with an internal ^{32}P and a 3'-terminal hydroxyl group.

Kinetics. All reactions were single-turnover, with ribozyme (E) in excess of radiolabeled substrate, and were carried out as previously described.[1b]

CHARACTERIZATION OF S_{Me}.

Mass spectrometry. The mass of S_{Me} was analyzed by MALDI-TOF mass spectrometry (calculated: 3357; found: 3360).

HPLC. S_{Me} and S_{ox} were characterized using reverse phase HPLC on a C_{18} column (Microsorb 300-5, Varian), using a linear gradient of acetonitrile (10-20% over 35

min) in 0.1 triethylammonium acetate (TEAA). S_{ox} eluted after 9.1 minutes (12.6% CH_3CN); S_{Me} after 10.2 minutes (12.9% CH_3CN). When mixed, the two substrates eluted in separate peaks in agreement with their retention times.

Alkaline hydrolysis. S_{Me} reacted when treated with 0.1 M NaOH at 50 °C for 1 hr Fig. 2, lanes 4 & 8), whereas no reaction was observed for S_{ox} in the same conditions Fig. 2, lanes 6 & 12). The reaction products for S_{Me} migrate on gel as expected for the reaction of a phosphonate diester (Scheme S1).

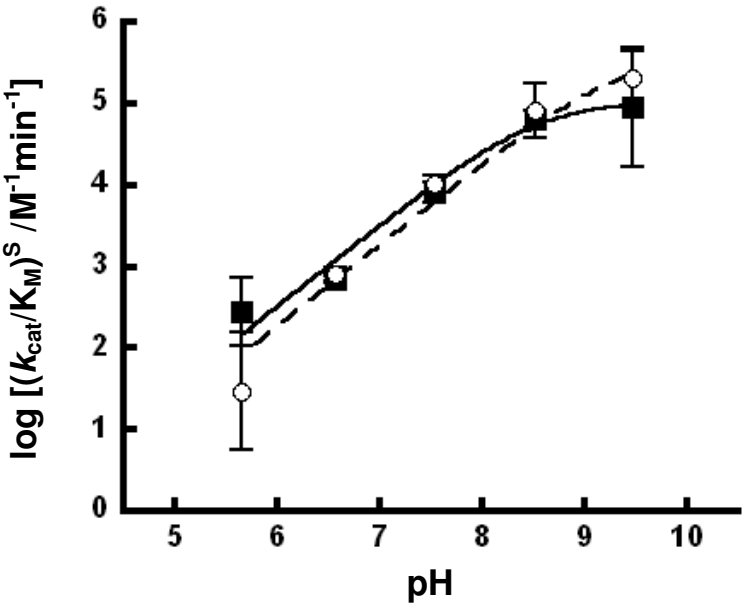
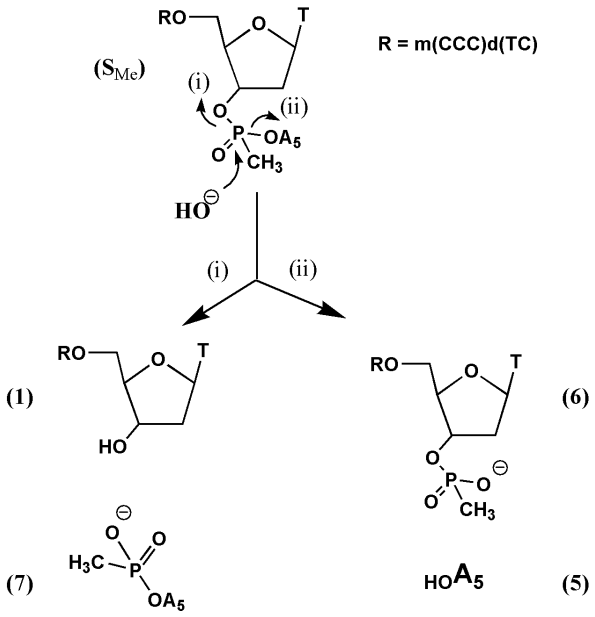


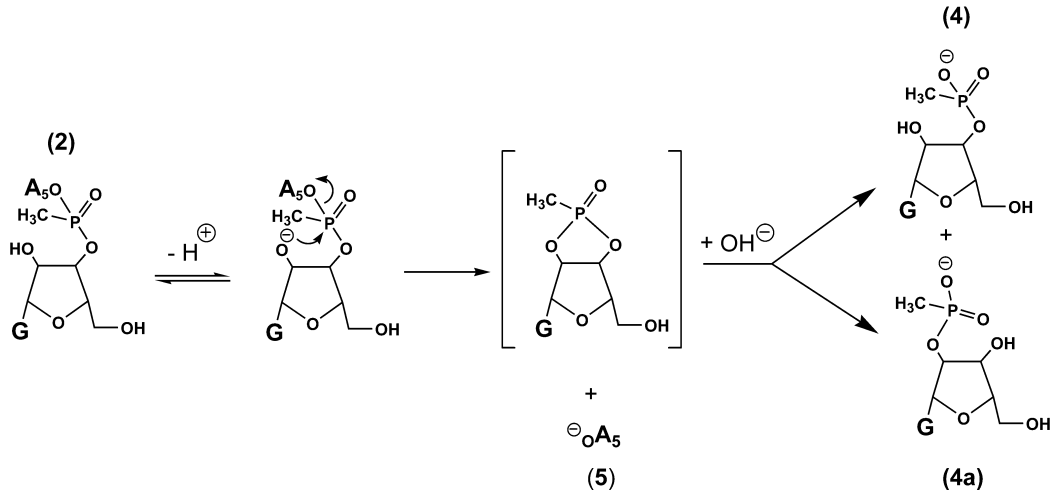
Figure S1. pH-rate profile of $(k_{cat}/K_M)^S$ for S_{Me} (●) and S_{ox} (○). The curvature above pH 8.5 presumably arises from denaturation of the ribozyme.[4]

Scheme S1



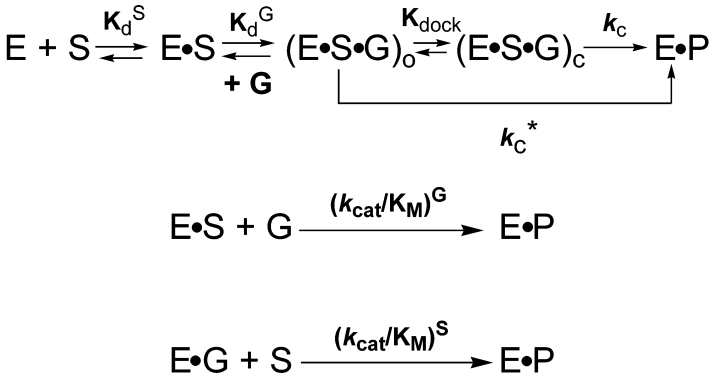
Products of alkaline hydrolysis of S_{Me} (0.1 M NaOH, 50 °C, 60 min.). Compound (**6**) presumably corresponds to the fast-migrating band in Figure 2, lane 4; similarly, compound (**7**) presumably corresponds to the fast-migrating product in Figure 2, lane 8. S_{ox} is stable in these conditions (Fig. 2, lanes 6 & 12).

Scheme S2



Complete reaction scheme for the spontaneous decomposition of (**2**).[5] Note that if (**2**) is radiolabeled at its 3'-end, only compound (**5**) is detected, as compounds (**4**) and (**4a**) remain unlabeled.

Scheme S3



Individual steps of the Tetrahymena ribozyme-catalyzed guanosine attack on phosphate esters. E, S, G and P are defined in the text. $(E•S•G)_o$ refers to an "open" complex, with only base-pairing interactions formed between E and S; $(E•S•G)_c$ refers to a "closed" complex, involving tertiary interactions between E and S.[6]

TABLE S1. Kinetic parameters, as defined in Scheme S3, for the *Tetrahymena* ribozyme-catalyzed reactions of S_{Me} and S_{ox}.

	S_{Me} [a]	S_{ox} [b]	ratio S_{Me}/S_{ox}
k_c^{*G} (/min^{-1})[c]	5.0 x 10^{-3}	6.7 x 10^{-3}	0.8
$(k_{cat}/K_M)^G$ (/M^{-1}min^{-1})[d]	4.2	6.3	0.7
K_d^G (/μM)[e]	800	800	(1)
$(k_{cat}/K_M)^S$ (/M^{-1}min^{-1})[f]	6.4 x 10^4	8.2 x 10^4	0.8
k_c^{*UCG} (/min^{-1})[g]	0.36 x 10^{-3}	2.2 x 10^{-3}	0.2
K_d^{UCG} (/μM)	24	21	1
k_c^{*G} (10 mm Mn^{2+})(/min^{-1})	0.25	0.062	4
k_c(-G) (/min^{-1})[h]	4.3 x 10^{-4}	0.21 x 10^{-4}	20
$k_c^{*G_{NH_2}}$ (/min^{-1})[i]	1.6 x 10^{-2}	0.44 x 10^{-2}	4

Reaction conditions: 30 °C, 450 nM E (unless otherwise stated), 10 mM Mg^{2+}, 50 mM NaEPPS, pH 8.3. [a] S_{Me} values are for the more reactive diastereoisomer. [b] Reactions of S_{ox} were carried out side-by-side with S_{Me}; the values reported agree within 3-fold with previous results.[7] [c] 2 mM G. [d] 0-2 mM G. [e] Solubility limits the maximum concentration of G that can be used to ~2 mM, and thus does not allow precise determination. [f] 2 mM G, 0-40 nM E. [g] 300 μM UCG. A competition experiment confirmed that G and UCG bind and react competitively. [h] This is the first order rate constant for the formation of products in absence of added nucleophiles, arising from a formal water attack on S. [i] 120 μM 2'-aminoguanosine (G_{NH_2}), 2 mM Mn^{2+}.[7b]

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