Probing General Base Catalysis in the Hammerhead Ribozyme

Jason M. Thomas and David M. Perrin*

Supporting Information:

Figure S1: MALDI-TOF spectra of (A) 5'-thiophosphorylated test sequence 5'-GTCTGTT ([M-H]$^-$ predicted mass: 2196.3; observed mass: 2196.9). (B) Sample from (A) after dephosphorylation by treatment with 25 mM NH_4OAc (pH 5) at 37°C for 1 hour (oligo-5'-SH [M-H]$^-$ predicted mass: 2116.4; observed mass: 2116.2). Peaks at +22, +44, etc. in (A) and (B) are sodium adducts. The 5'-thiophosphate is all but completely hydrolyzed by the pH 5 treatment, verifying that the thiophosphate was bonded to the 5'-carbon via sulphur. (The alternative thiophosphate attachment via oxygen would not be expected to quickly hydrolyze; and moreover would yield a 5'-hydroxy oligo (predicted mass: 2100.4)

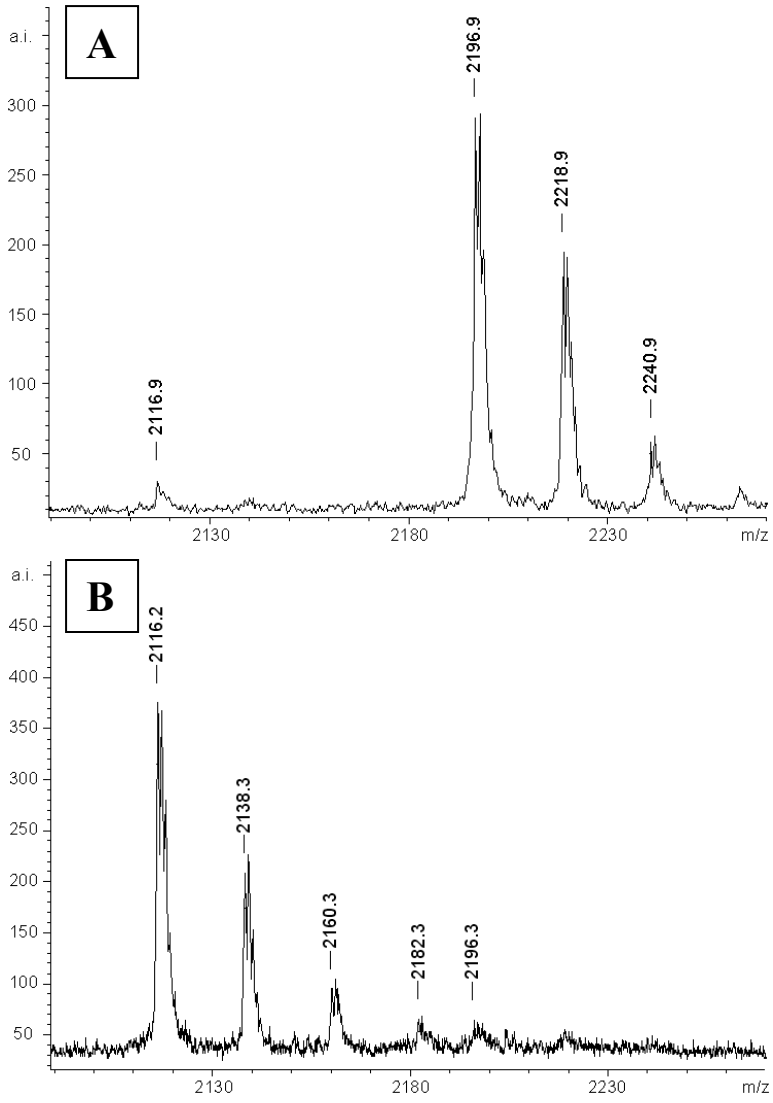
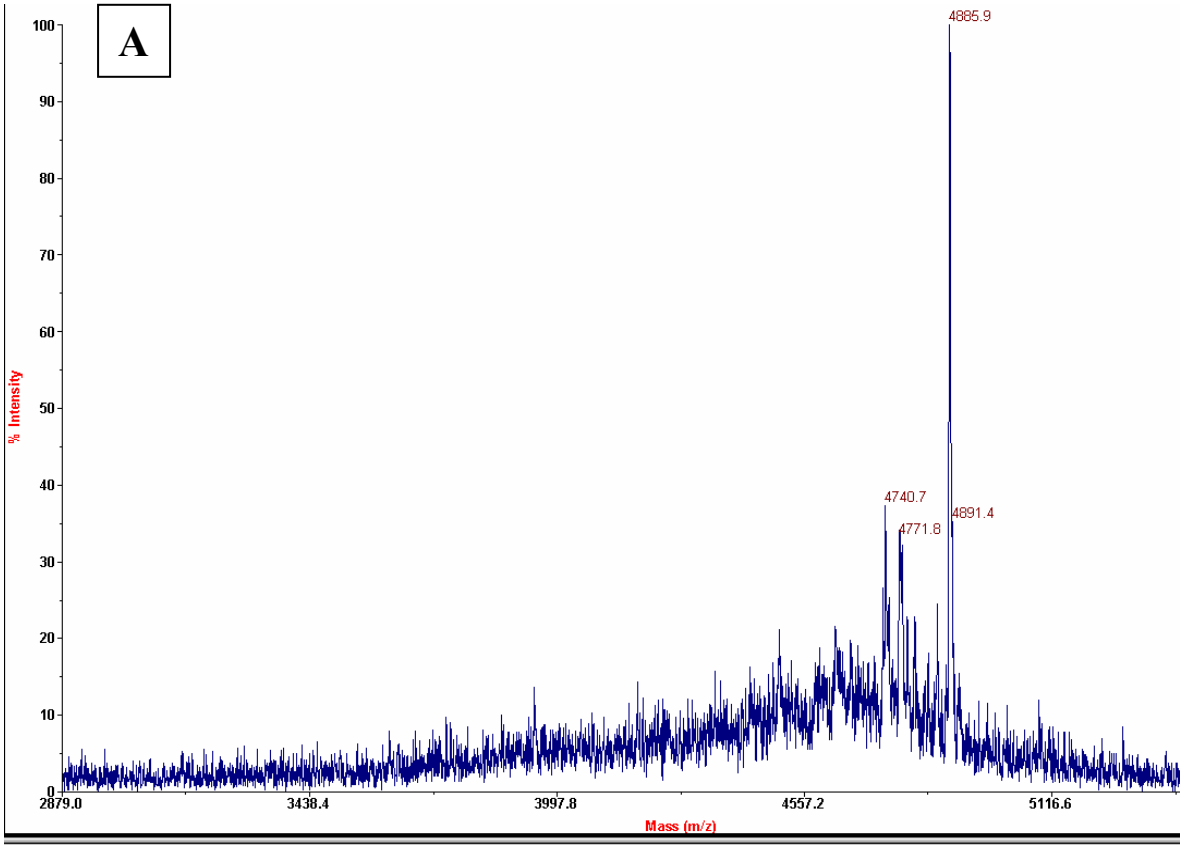


Figure S2: MALDI-TOF spectra of (A) 5'-thiophosphorylated 3'-product ($[M-H]^-$ predicted mass: 4885.8; observed mass: 4885.9). (B) Sample from (A) after dephosphorylation by treatment with 25 mM NH_4OAc (pH 5) at 37°C for 1 hour (oligo-5'-SH $[M-H]^-$ predicted mass: 4805.8; observed mass: 4803.7). Because of the greater length of these oligos compared to the test sequence characterized in Figure S1, MALDI-TOF spectra were much more difficult to obtain. Much higher laser power was required to obtain reasonable signal intensities. As a result, the mono-isotopic resolution seen in Figure S1 was not obtained, diminishing the mass accuracy. We suspect that this higher laser power is responsible for the artifactual peaks that were observed (namely masses 4740.7 and 4771.8 in (A)). The mass 4771.8 peak may result from fission of the 5'-carbon-sulphur bond, which would decrease the oligo mass by 133 (predicted oligo mass: 4772.8). We are unable to assign the minor peak with mass 4740.7. In (B), adducts with mass greater by ~101 were observed; we are unable to assign these, but suspect reaction of the 5'-thiol in some way with matrix fragments, perhaps generated during laser irradiation. A minor peak was also observed with mass 4787.5, close to that predicted for the 5'-hydroxy oligo (4789.8). We do not have an explaination for the appearance of this peak. The characterization data for the ligated S-link (Figure X) demonstrate that it contains only the 5'-bridging-thio linkage, so the appearance of this peak of speculative origin does not appear to be of significant concern for the exclusive synthesis of the bridging-thio linkage.



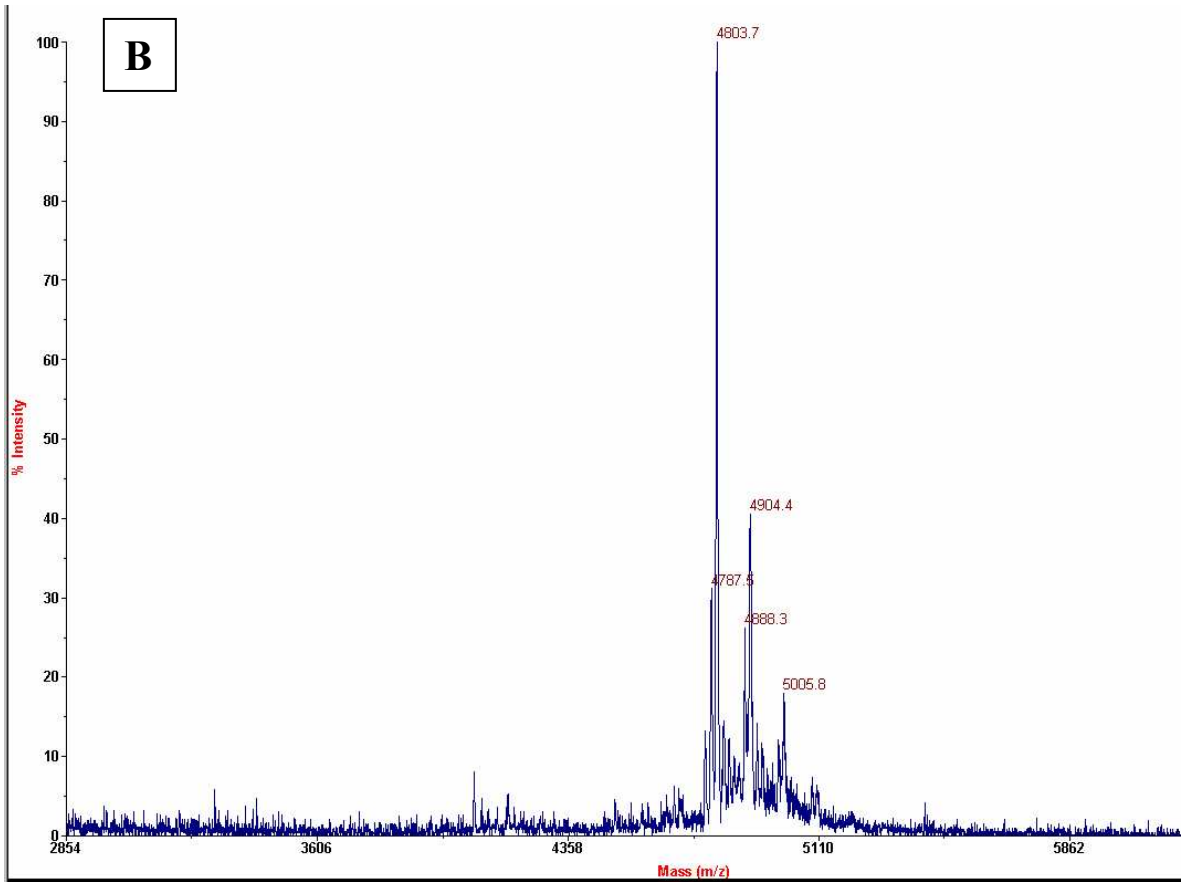
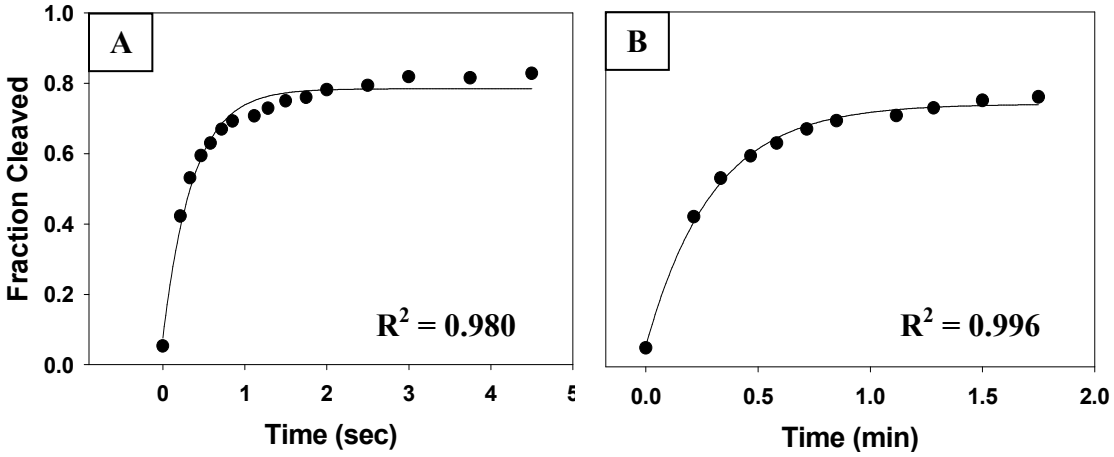

B
4803.7
4904.4
4787.5
4888.3
5005.8
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
2854
3606
4358
5110
5862

Figure S3: An example of biphasic cleavage kinetics encountered for cleavage of the S-link substrate the *S. mansoni* hammerhead ribozymes. The data for the single turnover cleavage of the S-link substrate by the WT ribozyme are shown (reaction conditions: 2 mM $MgCl_2$, 100 mM NaCl, 50 mM Na-PIPES pH 7). (A) The data are clearly biphasic when longer time points are included, and do not fit well ($R^2 = 0.980$) to a simple first order decay, given by $P = P_0 + P_\infty(1 - e^{-kt})$. (B) A truncated data set, where time points beyond the completion of the reaction of the fast cleaving population have been removed, but where enough data points are included to accurately fit the parameters which describe the fast cleaving phase ($R^2 = 0.996$). (C) The autoradiography data used to generate the plots in (A) and (B). Time points (in min) taken were: 0, 0.22, 0.33, 0.47, 0.58, 0.71, 0.85, 1.12, 1.28, 1.50, 1.75, 2.00, 2.50, 3.00, 3.75, 4.50.



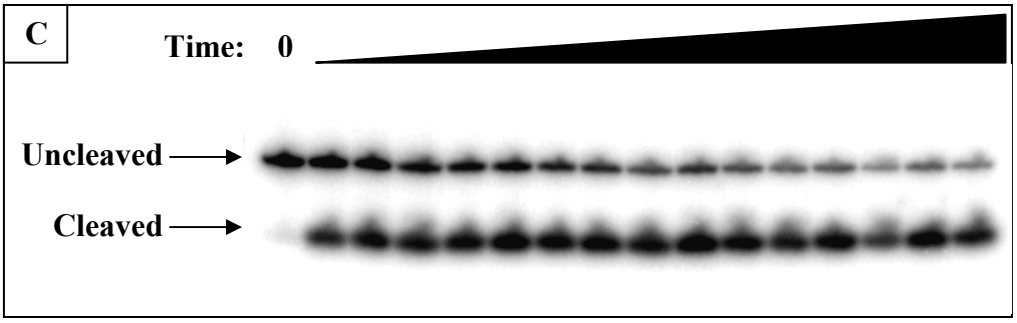
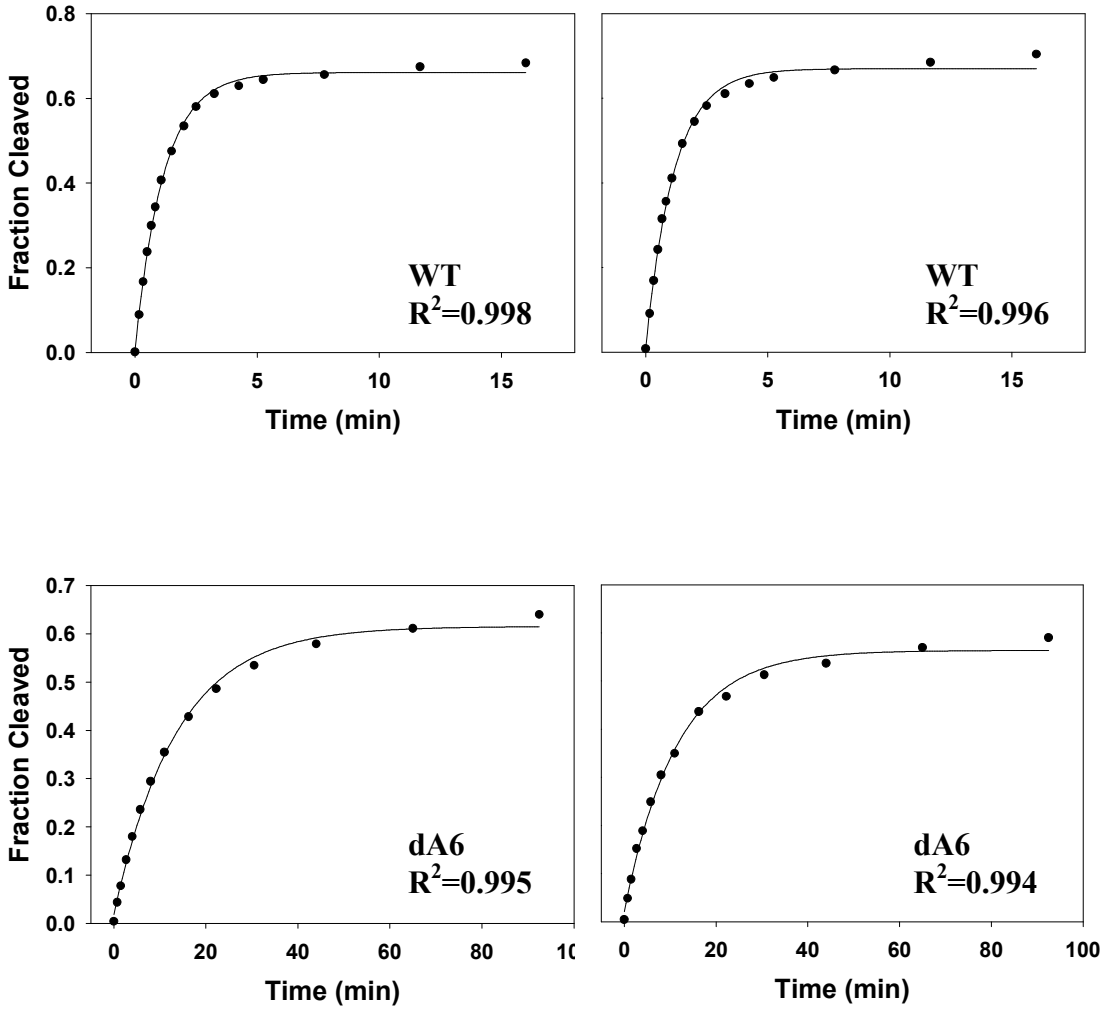


Figure S4: Examples of kinetic data for O-link substrate cleavage by the WT ribozyme and several mutants (two kinetic runs are shown for each of the indicated ribozymes). In all cases, the data were adequately described by a monophasic first order decay $P = P_0 + P_\infty(1 - e^{-kt})$. However, in all cases, longer time points consistently showed slightly more substrate cleavage than predicted by the monophasic trend line. Evidently, the rate constants for the two O-link cleavage phases are well separated such that much longer time points must be observed to fully characterize the slower cleaving phase. In contrast, the rate constants for the two S-link cleavage phases were more similar in magnitude; therefore, as shown in Fig. S3, the minor slower cleaving phase was more apparent near the end point of the faster cleaving phase. We did not characterize the slower cleaving phases for either of the O-link or S-link substrates because, invariably, most of the substrate was cleaved in the faster phase.

Our results are very similar to those reported by Pardi and co-workers. These authors reported biphasic cleavage kinetics for the *S. mansoni* hammerhead, where the slower phase rate constant was ~1000 times slower than the faster phase. (See: Canny, M. D.; Jucker, F. M.; Pardi, A. *Biochemistry* **2007**, *46*, 3826-3834)



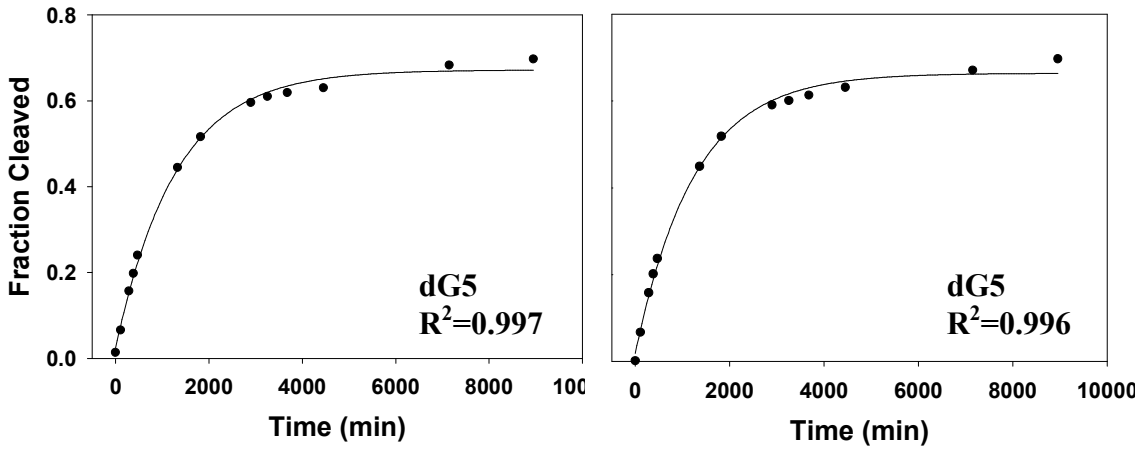


Figure S5: Comparison of the secondary structure representations of the wildtype and base paired ("BP") *S. mansoni* hammerhead ribozymes.