

Supporting Information:

Mechanistic Characterization of the HDV Genomic Ribozyme: A Mutant of the C41 Motif Provides Insight into the Positioning and Thermodynamic Linkage of Metal Ions and Protons

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S.1: Overview and Rationale:

In the Supporting Information we provide several kinetic models and equations that supplement those in the main text. The first set of equations (in **S.2**) is the complete set of parametric equations for Scheme 5. This is provided because these equations may be generally useful for treating pH-rate curves for ribozymes, and have not appeared previously; they are fully compatible with kinetic behavior of the genomic HDV ribozyme described in this manuscript, and previously (1). These equations show how the complex experimental behavior follows from an austere kinetic model.

The other two sets of kinetic models and equations for data fitting (Schemes 2' and 5' in sections **S.3** and **S.4** below) are derived from **GAB model 2**. Schemes 2' and 5' have just one additional kinetic step as compared to **GAB model 1** and are able to describe the data equally well. We present Schemes 2 and 5 (from the main text) alongside Schemes 2' and 5' for ease of comparison. It can be noted that Schemes 3 and 4 from the main text can also be extended to **GAB model 2** (not shown).

S.2: Single-Deprotonation/Single-Mg²⁺ Mechanism (GAB Model 1): Full Set of Parametric Equations (Scheme 5)

Scheme 5 from the main text (shown below) has four reactant states for the ribozyme: ligand-unbound, Mg²⁺-bound, H⁺-bound, and Mg²⁺- and H⁺-bound. This scheme led to eqs 3-6, in which eq 3 describes a general hyperbolic dependence of k_{obs} on Mg²⁺ that gives $K_{\text{d,obs}}$ and $k_{\text{max,obs}}$, and eqs 4-6 allow the two intrinsic Mg²⁺ affinity constants and two intrinsic pK_a values to be determined from the pH dependence of $K_{\text{d,obs}}$.

$$K_{\text{d,obs}} = K_{\text{d1}} \frac{1 + 10^{\text{p}K_{\text{a1}} - \text{pH}}}{1 + 10^{\text{p}K_{\text{a2}} - \text{pH}}} \quad (4)$$

$$K_{\text{d2}} = K_{\text{d1}} \times 10^{\text{p}K_{\text{a1}} - \text{p}K_{\text{a2}}} \quad (5)$$

$$k_{\text{max,obs}} = \frac{k_{\text{max}} K_{\text{a2}}}{K_{\text{a2}} + [\text{H}^+]} \quad (6)$$

We provide here a set of equations that lead to the same four intrinsic constants, but do so from k_{obs} -pH plots. The dependence of k_{obs} on pH can be fit to eq 1 to give pK_{a,obs} and $k_{\text{max,obs}}$ values, which are themselves parametric in Mg²⁺ concentration according to eqs 8 and 9.

$$k_{\text{obs,WI}} = \frac{k_{\text{max,obs}}}{1 + 10^{\text{p}K_{\text{a,obs}} - \text{pH}}} \quad (1)$$

$$\text{p}K_{\text{a,obs}} = \text{p}K_{\text{a2}} + \log\left(\frac{1 + K_{\text{d2}}/[\text{Mg}^{2+}]}{1 + K_{\text{d1}}/[\text{Mg}^{2+}]}\right) \quad (8)$$

$$k_{\text{max,obs}} = k_{\text{max}} \frac{[\text{Mg}^{2+}]}{K_{\text{d1}} + [\text{Mg}^{2+}]} \quad (9)$$

Secondary plots of pK_{a,obs} as a function of Mg²⁺ (eq 8), in combination with eq 5, can be used to provide the four intrinsic constants. This set of equations describes a set of log k_{obs} -pH plots that have the same Mg²⁺-dependencies for pK_{a,obs} and $k_{\text{max,obs}}$ as observed previously (1).

S.3: Single-Protonation/Single-Deprotonation Mechanism (GAB Model 2): Kinetic Model and Equations (Scheme 2')

Scheme 2 from the main text (shown below) has two reactant states of the ribozyme: H⁺-bound and H⁺-free. This scheme led to eq 1, which describes a general dependence of k_{obs} on pH and gives $\text{p}K_{\text{a,obs}}$ and $k_{\text{max,obs}}$ values, which are themselves a function of Mg^{2+} as described by eqs 8 and 9. The rate law in eq 1 involves a single deprotonation and is the rate equation commonly associated with general base catalysis. We provide here an equation that leads to the same $\text{p}K_{\text{a,obs}}$ and asymptotes as eq 1, but does so through a single-protonation/single-deprotonation mechanism.

Schemes 2 and 2' are shown below. As can be seen in the side-by-side comparison, the Schemes differ only in which of the two reactant states are functional. In Scheme 2, which reacts according to **GAB model 1**, it is the right-hand nucleobase-deprotonated, fully-hydrated-magnesium reactant state that is functional to carry out chemistry. In Scheme 2', which reacts according to **GAB model 2**, an additional reactant state is connected to the left-hand nucleobase-protonated state. This state shows ionization of the fully hydrated magnesium ion to the pentahydrated magnesium hydroxide, and it is this reactant state that is functional to carry out chemistry. Given that the $\text{p}K_{\text{a}}$ of the hydrated magnesium ion is 11.4 (2), this last ionization is not especially favorable near neutrality. Thus, the fraction of magnesium ions in the hydroxide state is well-approximated by $10^{\text{pH}-11.4}$. This leads to the following modified version of eq 1:

$$k_{\text{obs,WT}} = \frac{k_{\text{max,obs}} 10^{\text{pH}-11.4}}{1 + 10^{\text{pH}-\text{p}K_{\text{a,obs}}}} \quad (1')$$

Here, the asymptote of a k_{obs} -pH plot is given by $k_{\text{max,obs}} 10^{\text{p}K_{\text{a,obs}}-11.4}$.

S.4: Single-Protonation/Single-Deprotonation/Single-Mg²⁺ Mechanism (GAB Model 2):

Kinetic Model and Full Set of Parametric Equations (Scheme 5')

Scheme 5' is a hybrid of Schemes 5 and 2'. It is apparent in the side-by-side comparison of Schemes 5 and 5' that the reactant state has moved from bottom right-hand state in Scheme 5 to the bottom left-hand state. As in going from Scheme 2 to 2', an additional reactant state is connected to the left-hand nucleobase-protonated state in Scheme 5'. This leads to the following set of parametric equations:

Set A: Dependence of k_{obs} on Mg²⁺ (parametric in pH)

Eq 4' is the same as eq 4 (4')

Eq 5' is the same as eq 5 (5')

$$k_{\text{max,obs}} = \frac{k_{\text{max}} 10^{-11.4}}{K_{a2} + [\text{H}^+]} \quad (6')$$

Set B: Dependence of k_{obs} on pH (parametric in [Mg²⁺])

Eq 8' is the same as eq 8 (8')

$$k_{\text{max,obs}} = k_{\text{max}} 10^{\text{p}K_{a2} - 11.4} \frac{[\text{Mg}^{2+}]}{K_{d1} + [\text{Mg}^{2+}]} \quad (9')$$

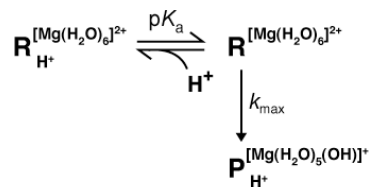
As described in the text, one of the fundamental differences between Schemes 5 and 5' is that in Scheme 5' there is an electrostatic repulsion in the chemically competent state, which is relieved upon reaction, suggesting that ground state destabilization could be a driving force for **GAB model 2** (1, 3).

References

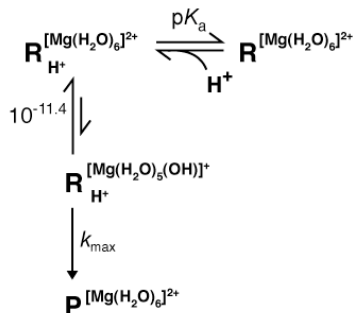
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Supporting Schemes:¹

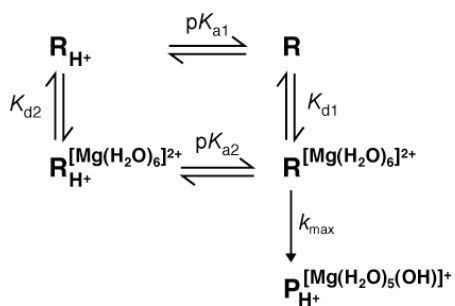
Scheme 2. Single-Deprotonation Mechanism (GAB Model 1)



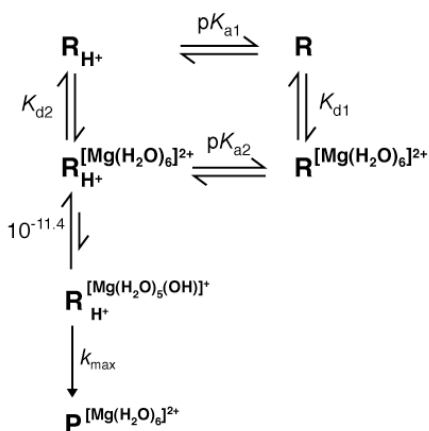
Scheme 2'. Single-Protonation/Single-Deprotonation Mechanism (GAB Model 2)



Scheme 5. Single-Deprotonation/Single-Mg²⁺ Mechanism (GAB Model 1)



Scheme 5'. Single-Protonation/Single-Deprotonation/Single-Mg²⁺ Mechanism (GAB Model 2)



¹ Schemes 2 and 5 are reproduced from the main text to facilitate comparison **GAB models 1 and 2.**