Supporting Information

A catalytic metal ion interacts with the cleavage site G•U wobble in the HDV ribozyme[†]

Jui-Hui Chen[‡⊥], Bo Gong[§⊥], Philip C. Bevilacqua[∥], Paul R. Carey[§*], and Barbara L. Golden[‡*]*

Running title: A catalytic Mg^{2+} ion in the HDV ribozyme

[‡]Department of Biochemistry, Purdue University, 175 South University Street, West Lafayette, Indiana 47907, and [§]Department of Biochemistry, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, and [∥]Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, Pennsylvania 16802.

[†]This project was supported by NSF Grant MCB-0527102 to PCB and NIH Grant GM-54072 to PRC, the Purdue University Department of Biochemistry and the Purdue University Cancer Center.

*To whom correspondence should be addressed: Barbara Golden, Tel. (765) 496-6165, Fax. (765) 494-7897, E-mail: barbgolden@purdue.edu; and Paul Carey, Tel. (216) 368-0031; Fax. (216) 368-3419; E-mail: prc5@cwru.edu; and Philip Bevilacqua: Tel. (814) 863-3812 ; Fax. (814) 863-8403; E-mail: pcb@chem.psu.edu.

[⊥]These authors contributed equally to this work.

SUPPLEMENTARY MATERALS AND METHODS

The basis for the assignments in the HDV Raman difference spectrum shown in Figure 4. The assignments are consonant with our recent work (*1*) and are supported by Figure S2 that shows the Raman difference spectrum of CMP, pH 6.0 minus pH 3.0. The modes for neutral (C) and protonated (C^+) CMP are also listed in Table S1 and a direct correspondence between the features from CMP and HDV can be seen at 1528, 1298, 780 and 599 cm^{-1} (C) and 1258 and 790 cm^{-1} (C^+). Based on other recent work (*2*), the positive feature at 323 cm^{-1} in Figure 1 is assigned to a magnesium hydrate species $Mg^{2+}(H_2O)_x$ ($x \leq 5$) that is bound to (an) inner sphere ligand(s). Where, likely, the tetra- or penta- hydrate has two or one exogenous ligand from RNA groups, respectively. In addition, based on literature data (*3*) and unpublished data the feature at 1489 is assigned to G and these at 1341 and 728 cm^{-1} are assigned to A ring modes (*4*).

Quantum mechanical calculations of pentahydrate Mg^{2+} bound inner-sphere to N7 of a guanine. Quantum mechanical calculations were performed to study the inner-sphere interactions between a pentahydrate Mg^{2+} ($Mg(5H_2O)^{2+}$) and N7 of a guanine using Gaussian 03 software. Calculations were carried out at the B3LYP/6-31+G(d) level as described previously (*2*). The model used in present calculation is shown in Figure S2 and the geometry optimization was done during the calculation. The calculation for a free guanine revealed that N7-C8 stretching of guanine makes the most contribution to the band at 1496 cm^{-1} (calculated value, corresponding to the experimental value of 1483 cm^{-1}). This observation is in very good agreement with previous work done by Majoube (*5*). The formation of inner-sphere interaction of $[Mg(H_2O)_5]^{2+}$ — N7-guanine upshift 1496 cm^{-1} band to 1501 cm^{-1} (calculated values). This upshift is consistent with the experimental observation which showed the peak at 1483 upshifts to 1489 cm^{-1} upon Mg^{2+} binding. The agreement between calculations (~5 cm^{-1} upshift) and experiment (~6 cm^{-1} upshift) supports previous assumptions that the differential peak around 1480 cm^{-1} region upon magnesium binding, observed in Figure 4a, is due to direct coordination of the metal ion to N7 of guanine (*6*).

Table S1

Positions (cm^{-1}) and assignment of major Raman difference bands observed in Raman difference spectra of [HDV pH 7.5] minus [HDV pH 5.0] and [CMP pH 6.0] minus [CMP pH 3.0]

HDV		CMP	
Wavenumber / cm^{-1}	assignment	Wavenumber / cm^{-1}	assignment
		1547	C^+
1528	C	1529	C
1489/1473	G		
1341	A		
1298	C	1298	C
1258	C^+	1258	C^+
		983	PO_3^{2-}
		929	ribose
916/896	ribose		
		892	ribose
815	-O-P-O-		
790	C^+	790	C^+
780	C	778	C
728	A		
599	C	599	C
323	$Mg(H_2O)_x^{2+}$ (x≤5)		

C, neutral cytosine; C^+, protonated cytosine; $Mg(H_2O)_x^{2+}$ (x≤5), inner-sphere coordinated Mg-hydrate. The features assigned to the bases are due to ring modes.

Figure S1. Sequence of the HDV ribozyme used in this study.

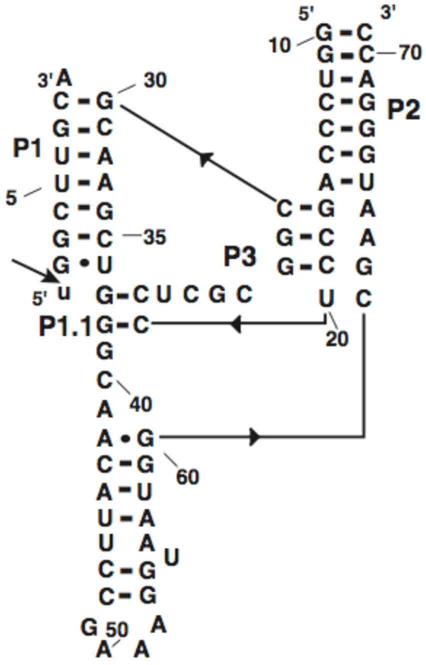
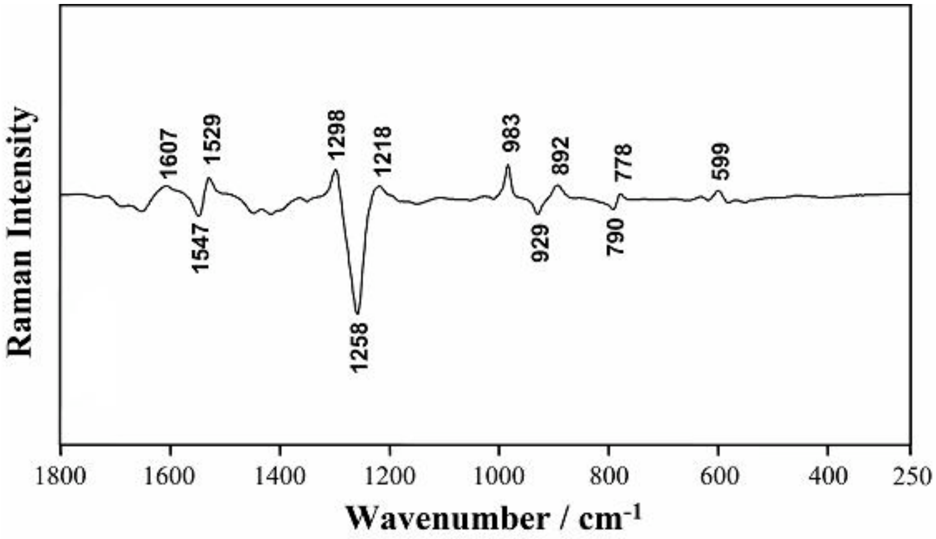


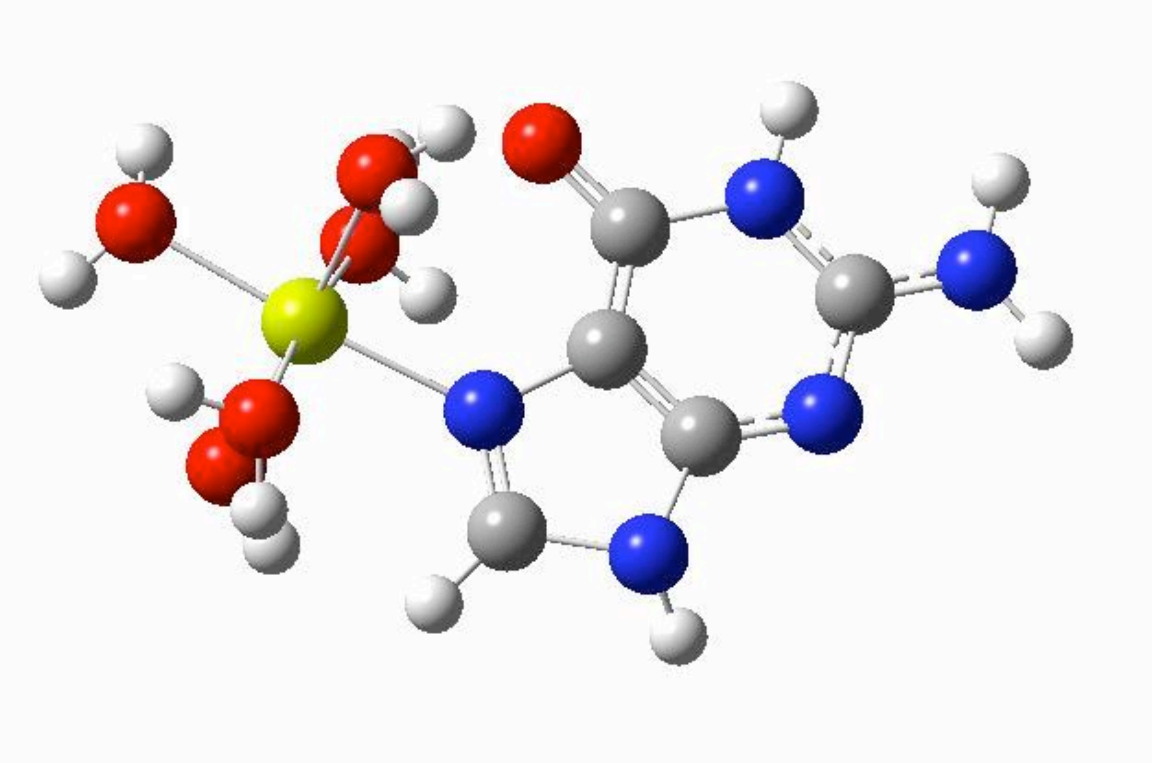
Figure S2. Raman difference spectrum of [CMP pH 6.0] minus [CMP pH 3.0]



Experimental details see ref 1. The difference peaks represent the changes of vibrational modes of cytidine and phosphate group due to the neutral CMP (positive peaks) and protonated CMP (negative peaks).

Figure S3

Model used in quantum mechanical calculations of a pentahydrate Mg^{2+} bound inner-sphere to N7 of a guanine. Colors of atoms: Mg^{2+} (yellow); Oxygen (red); Nitrogen (blue); Hydrogen (grey).



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