

Detection of Inner-sphere Interactions between Magnesium Hydrate and the Phosphate Backbone of the HDV Ribozyme Using Raman Crystallography

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Supporting Information

The Raman spectra of aqueous $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$. Figure S1 shows the low frequency region 3 M MgCl_2 in H_2O , D_2O and H_2^{18}O . The ν_1 mode occurs at 360, 341 and 341 cm^{-1} , respectively. The observed 341 cm^{-1} band in D_2O is in agreement with the expected isotope shift calculated as:

$$\nu_1[\text{Mg}(\text{OD}_2)_6^{2+}] = (18.02/20.03)^{1/2} \times \nu_1[\text{Mg}(\text{OH}_2)_6^{2+}] = 0.948 \times 360 \text{ cm}^{-1} = 341 \text{ cm}^{-1}$$

This equation uses the simple formula $\nu = (1/2\pi)(k/\mu)^{1/2}$. Where ν is frequency, k is force constant invariant for each isotope, and μ is reduced mass (reference 12(a), chapter 2).

Quantum mechanical calculations using Gaussian. *Ab initio* quantum mechanical calculations were performed to predict the Raman spectra of $(\text{Mg}\cdot 6\text{H}_2\text{O})^{2+}$, $(\text{Mg}\cdot 5\text{H}_2\text{O})^{2+}(\text{H}_2\text{PO}_4)^-$ and $(\text{Mg}\cdot 4\text{H}_2\text{O})^{2+}2(\text{H}_2\text{PO}_4)^{2-}$ using Gaussian 03. Calculations were performed at the DFT level using

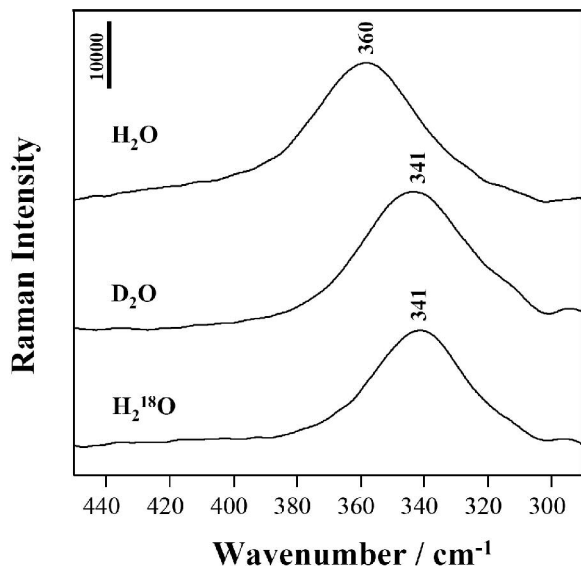


Figure S1. Raman feature for the symmetric stretch (A_{1g}) of magnesium hexahydrate (3 M) in H_2O , D_2O and H_2^{18}O .

the 6-31+G(d) basis set. DFT calculations were performed with Becke's three-parameter hybrid method using the correlation functional of Lee, Yang, and Parr (B3LYP) (with 20% Hartree-Fock exact exchange mixing).

The calculations (unscaled) for a single molecule of $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$ *in vacuo* gave values of 317, 300 and 300 cm^{-1} for the unsubstituted, the D-substituted, and the ^{18}O -substituted molecules, respectively. The corresponding experimental values are 360, 341 and 341 cm^{-1} , respectively. The agreement between experiment and calculations is only approximate, in part due to use of a single $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$ species to model the form in solution whereas the hexahydrate is actually surrounded by a complex water structure. However, the trend in the isotope shifts is reproduced satisfactorily. The isotope shift 17 cm^{-1} ($= 317 - 300 \text{ cm}^{-1}$) is very close to the observed and calculated shifts of 19 cm^{-1} , ($= 360$

– 341 cm^{-1}), above.

For the calculations on the magnesium pentahydrate- PO_2^- complex the following protocol was observed. Using the $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$ as an octahedral template, one H_2O was replaced by H_2PO_4^- with one of the non-protonated phosphoryl oxygens bonding to Mg^{2+} . The geometry of the complex was optimized during the calculation. The calculations for magnesium pentahydrate - PO_2^- complex *in vacuo* gave values of 295, 282 and 282 cm^{-1} for the unsubstituted, the D-substituted, and the ^{18}O -substituted molecules, respectively. Again the isotope shifts reproduce the experimental values fairly well and the difference in the absolute values of the modes likely reflects, in part, the fact that the calculations are performed *in vacuo*. We also calculated two possible arrangement of magnesium tetrahydrate- $[\text{PO}_2^-]_2$ complex *in vacuo*, with two PO_2^- groups either at *cis* or *trans* positions of the octahedral template. The results gave values of 279 and 290 cm^{-1} for *cis* and *trans* magnesium tetrahydrate- PO_2^- complexes, respectively, the latter is very close to the calculated value of magnesium pentahydrate- PO_2^- complex.

Interaction between magnesium hydrate and dimethyl phosphate in aqueous solution. At very high concentrations of aqueous dimethyl phosphate and magnesium hexahydrate there is Raman spectroscopic evidence for inner-sphere complexes involving the model compound's PO_2^- group and $[\text{Mg}(\text{H}_2\text{O})_5]^{2+}$. In the Raman spectrum a solution of 0.2 M dimethyl phosphate and 1 M MgCl_2 the “ PO_2^- derivative” occurs at 1107 and 1084 cm^{-1} while a feature due to $[\text{Mg}(\text{H}_2\text{O})_5]^{2+}$ is seen at 321 cm^{-1} (Figure S2). Since the association between the phosphate and magnesium is very weak, the complexes giving rise to the Raman difference features must contain the pentahydrate. It is improbable that more than one phosphate will enter the inner-sphere at a given instant. These model experiments will be discussed in detail in a future publication.

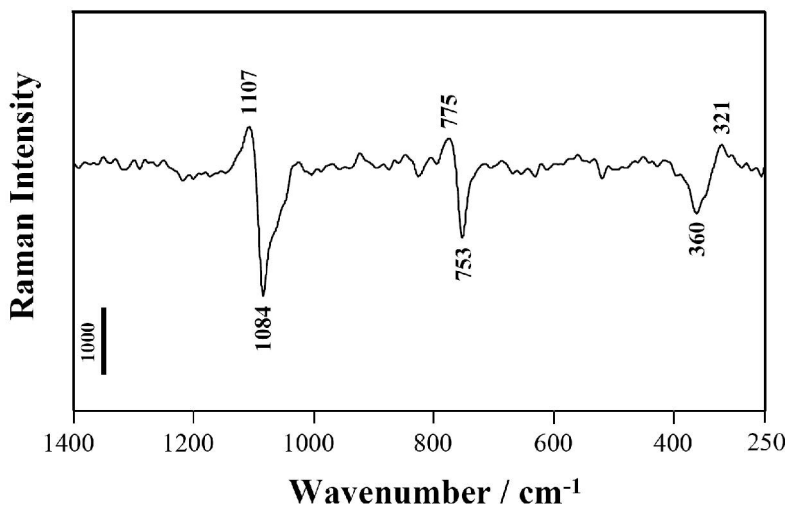


Figure S2. Raman difference spectrum [200 mM dimethyl phosphate + 1 M MgCl_2] minus [200 mM dimethyl phosphate]. The background spectrum of 1 M MgCl_2 has also been subtracted to remove the large contribution due to $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$ that reveals the small negative feature at 360 cm^{-1} . The fact that the negative feature is small is indicative of the aforementioned very weak nature of the interaction.

Estimating the number of PO_2^- groups inner-sphere coordinated to Mg^{2+} ions in HDV.

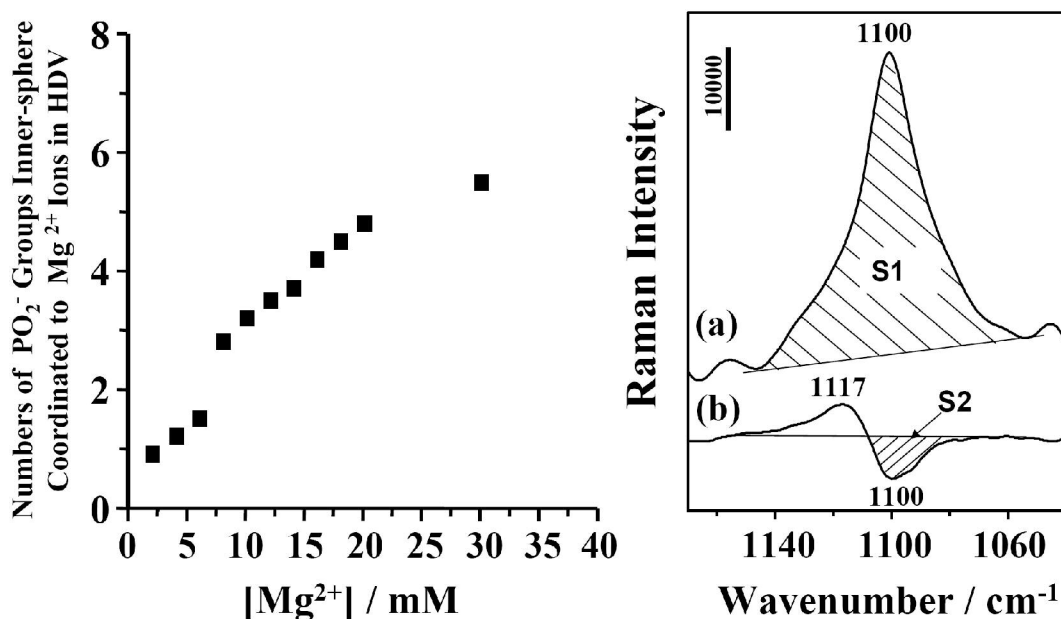


Figure S3. Semi-quantitative analysis of numbers of PO_2^- groups inner-sphere coordinated to Mg^{2+} ions in HDV by Raman crystallography (left). The number is calculated based on the changes of integrated area of $-\text{PO}_2^-$ peak at 1100 cm^{-1} seen in the difference spectrum due to their removal by Mg^{2+} - PO_2^- interactions, ratioed to the parent spectrum. In right hand panel, (a) shows the original PO_2^- peak of HDV in the absence Mg^{2+} while (b) gives the difference spectrum of $[\text{HDV} + 20 \text{ mM Mg}^{2+}]$ minus $[\text{HDV no Mg}^{2+}]$. Thus, the numbers of $-\text{PO}_2^-$ coordinated to Mg^{2+} is obtained using the equation: $N_{\text{PO}_2^-} = (S2/S1) \times 71$, where $N_{\text{PO}_2^-}$ is the number of $-\text{PO}_2^-$ inner-sphere coordinated to Mg^{2+} per HDV molecule; S1 and S2 represent the integrated areas of original $-\text{PO}_2^-$ peak, and peak change caused by the $-\text{PO}_2^-$ group interacting with the Mg^{2+} ion, respectively; 71 is the total number of phosphate groups of the HDV ribozyme in our RNA sequence.

Mg^{2+} binding sites in the crystal structure of HDV. Figure 1 (main text) shows a rendering of pdb entry 1cx0 highlighting the RNA, the U1A protein binding loop, and the 12 magnesium ions identified by X-ray crystallography. The magnesium ions are spheres. There are 3 Mg^{2+} s near the U1A protein and the U1A binding loop. These will clearly not be present in the crystals used in this analysis as no protein is present in these crystals. This leaves 9 magnesium ions binding purely through ribozyme ligands. Of these, one is binding to the tether that connects the substrate to the ribozyme. This is also not present in the present crystals, and we would not expect to see it. Another is on a crystal contact. These crystal contacts are unique to individual crystal forms and again are not expected to be seen in the crystals with which we are working. This leaves 7 magnesium ions bound in the major grooves and the core of the molecule, 5 of which make potential inner-sphere $-\text{PO}_2^-$ - Mg^{2+} contacts based on the contacts of Juneau and co-workers¹⁵ (Table S1). The RNA used in this study has a slightly different sequence, and contains the scissile phosphate. Thus, it is likely that the magnesium ions in the major grooves will bind in slightly different places. However, this analysis of pdb entry 1cx0 suggests that it would not be

unreasonable to expect ~7 metal ions to be bound to an RNA of this size with ~5 Mg²⁺ ions engaged in inner-sphere contacts with phosphate. Table S1 shows all the Mg²⁺-base and Mg²⁺-PO₂⁻ contacts.

Table S1: Mg²⁺ binding sites in HDV crystal structure from pdb (1cx0).

	Nucleotide	Atom	Distance (Å)
Mg402	G170	O2P	5.2
	G170	O6	2.8
	G170	N7	2.5
Mg404	G138	O6	4.7
	G138	N7	3.2
	G164	O1P	3.7
Mg405	G105	O2P	6.5
	G105	O6	3.5
	G105	N7	4.2
Mg407	G117	O1P	2.8
	G117	O2P	4.3
	C118	O1P	4
	C118	O2P	2.8
Mg408	C119	O2P	2.6
	U120	O2P	4.4
Mg411	G135	O1P	3.2
	G135	O2P	4.6
	C136	O1P	3.6
	C136	O2P	2.8
Mg412	U123	O1P	3.76
	C124	O1P	3.32
	C124	O2P	2.66

Magnesium to phosphate oxygen distances 2.8 Å or less are highlighted in red.⁶