Assessing the Potential Effects of Active Site Mg$^{2+}$ Ions in the *glmS* Ribozyme–Cofactor Complex

Sixue Zhang, David R. Stevens, Puja Goyal, Jamie L. Bingaman, Philip C. Bevilacqua, and Sharon Hammes-Schiffer

Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801-3364, United States
Department of Chemistry and Center for RNA Molecular Biology and Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, United States

Supporting Information

**ABSTRACT**: Ribozymes employ diverse catalytic strategies in their self-cleavage mechanisms, including the use of divalent metal ions. This work explores the effects of Mg$^{2+}$ ions in the active site of the *glmS* ribozyme–GlcN6P cofactor complex using computational methods. Deleterious and potentially beneficial effects of an active site Mg$^{2+}$ ion on the self-cleavage reaction were identified. The presence of a Mg$^{2+}$ ion near the scissile phosphate oxygen atoms at the cleavage site was determined to be deleterious, and thereby anticatalytic, due to electrostatic repulsion of the cofactor, disruption of key hydrogen-bonding interactions, and obstruction of nucleophilic attack. On the other hand, the presence of a Mg$^{2+}$ ion at another position in the active site, the Hoogsteen face of the putative base, was found to avoid these deleterious effects and to be potentially catalytically favorable owing to the stabilization of negative charge and pK$_a$ shifting of the guanine base.

Ribozymes are RNA molecules that catalyze specific reactions in a wide range of organisms. In contrast to proteins, ribozymes have high negative charges due to the phosphate groups in the backbone, resulting in the participation of metal ions for structural stabilization and, in some cases, catalysis. While metalloproteins usually use transition metals as additional techniques. In the context of this Letter, we consider the role of metal ions in the *glmS* ribozyme.

In contrast to the self-cleavage mechanisms of other small ribozymes, the *glmS* ribozyme employs an exogenous cofactor, glucosamine-6-phosphate (GlcN6P), in its self-cleavage reaction and utilizes the protonated cofactor as the general acid in the acid–base mechanism. For this ribozyme, divalent metal ions such as Mg$^{2+}$ and Ca$^{2+}$ have been shown to influence the rate and overall apparent pK$_a$ of the self-cleavage reaction. According to a previously proposed mechanism, A-1(O2$^-$) attacks the scissile phosphate in conjunction with deprotonation of this O2$^-$ by G40(N1), which was presumed to be deprotonated by an external base and may be activated by a metal ion. In addition, the leaving group G1(O5$^-$) is protonated by the amine group of the cofactor (Figure 1; see Figure S2 for the secondary structure).

In this Letter, we examine several possible Mg$^{2+}$ binding sites with computational methods. Deleterious and potentially catalytic effects of Mg$^{2+}$ ions in the active site were explored using both classical molecular dynamics (MD) and mixed quantum mechanical/molecular mechanical (QM/MM) approaches. Minimum free energy paths and relative free energies of different configurations were determined using the finite temperature string method combined with umbrella sampling and pK$_a$ calculations were performed using Poisson–Boltzmann calculations with a linear response approach (PB/LRA). The details of the computational methods are provided in the Supporting Information. Analysis of the potentially catalytic or deleterious (i.e., anticatalytic) effects of Mg$^{2+}$ ions in the active site provides insights into the self-cleavage mechanism of the *glmS* ribozyme and also contributes to the general understanding of small ribozyme catalysis.

To explore potential roles of active site Mg$^{2+}$ ions, we examined several possible Mg$^{2+}$ binding sites with computational techniques. In the first scenario, the effects of a Mg$^{2+}$ ion containing the GlcN6P cofactor in the precleavage state (PDB ID 2Z7S). In this crystal structure, which at 1.7 Å is high-resolution for RNA, self-cleavage was inhibited by a deoxy at the cleavage site. No Mg$^{2+}$ ions in the active site were resolved, but nine other Mg$^{2+}$ ions were resolved (Figure 1A) and remained near their original positions during the simulations. In addition to these crystallographic Mg$^{2+}$ ions, we manually added one Mg$^{2+}$ ion to the active site at different positions to test possible roles in catalysis using both classical molecular dynamics (MD) and mixed quantum mechanical/molecular mechanical (QM/MM) approaches. Minimum free energy paths and relative free energies of different configurations were determined using the finite temperature string method combined with umbrella sampling and pK$_a$ calculations were performed using Poisson–Boltzmann calculations with a linear response approach (PB/LRA). The details of the computational methods are provided in the Supporting Information. Analysis of the potentially catalytic or deleterious (i.e., anticatalytic) effects of Mg$^{2+}$ ions in the active site provides insights into the self-cleavage mechanism of the *glmS* ribozyme and also contributes to the general understanding of small ribozyme catalysis.

Received: August 17, 2016
Accepted: September 14, 2016
Published: September 28, 2016

DOI: 10.1021/acs.jpclett.6b01854
J. Phys. Chem. Lett. 2016, 7, 3984–3988
The Mg\textsuperscript{2+} ions in the crystal structure are represented by pink spheres. (B) Illustration of the active site in the glmS ribozyme and the proposed self-cleavage mechanism indicated by the red arrows. The pro-R\textsubscript{P} and pro-S\textsubscript{P} oxygens of the scissile phosphate are labeled with subscripts R and S, respectively. Dashed black lines indicate important hydrogen bonds in the active site. The potential positions of the active site Mg\textsuperscript{2+} ion examined in this Letter are indicated by dotted circles. They are denoted “Site 1”, which is near the nonbridging oxygens of the scissile phosphate, and “Site 2”, which is near the Hoogsteen face of G40.

In an effort to understand the absence of a divalent metal ion at the active site of the holoribozyme, we examined two representative cases for the presence of a Mg\textsuperscript{2+} ion at Site 1, the cleavage site for the holoribozyme. In one case, the Mg\textsuperscript{2+} ion was coordinated to the pro-S\textsubscript{P} oxygen. To test the mobility of this Mg\textsuperscript{2+} ion, we conducted a classical free energy simulation with the string method combined with umbrella sampling. This simulation was performed for the canonical state, in which both A-1 and G40 are in their canonical protonation states, as shown in Figure 1B. The calculated minimum free energy path indicated that the free energy barrier associated with the movement of the Mg\textsuperscript{2+} ion from one nonbridging oxygen to the other is \(~3\text{–}5\) kcal/mol, with another stable configuration midway between the two oxygen atoms, and that the free energy difference between these equilibrium configurations is less than 1 kcal/mol (Figure S3). Thus, the Mg\textsuperscript{2+} ion was able to move from one nonbridging oxygen of the scissile phosphate to the other under the simulation conditions used in this work.

Table 1. Average Active Site Distances from Classical MD Simulations with a Mg\textsuperscript{2+} Ion at the Cleavage Site

| species                  | Mg\textsuperscript{2+} position | GlcN6P(O1):pro-R\textsubscript{P} | A-1(I’O2’):G40(N1) |
|--------------------------|----------------------------------|-----------------------------------|---------------------|
| canonical w/o Mg\textsuperscript{2+} | n/a                             | 2.66 (0.10)            | 3.20 (0.28)         |
| canonical Mg\textsuperscript{2+}@S\textsubscript{P} | 1.96 (0.05)/4.08 (0.19)       | 4.39 (0.74)            | 5.16 (1.95)         |
| canonical Mg\textsuperscript{2+}@R\textsubscript{P} | 4.03 (0.21)/1.94 (0.04)        | 6.04 (1.62)            | 4.91 (0.83)         |
| dO2’ w/o Mg\textsuperscript{2+}           | n/a                             | 2.51 (0.08)            | 2.93 (0.19)         |
| dO2’ Mg\textsuperscript{2+}@S\textsubscript{P} | 1.96 (0.06)/4.03 (0.32)       | 2.56 (0.09)            | 6.12 (1.17)         |
| dO2’ Mg\textsuperscript{2+}@R\textsubscript{P} | 4.32 (0.36)/1.96 (0.05)        | 2.83 (0.12)            | 7.47 (1.55)         |
| dN1 w/o Mg\textsuperscript{2+}            | n/a                             | 2.71 (0.09)            | 2.88 (0.11)         |
| dN1 Mg\textsuperscript{2+}@S\textsubscript{P} | 1.96 (0.05)/4.22 (0.16)       | 3.82 (0.19)            | 4.60 (1.79)         |

“The cleavage site is labeled as Site 1 in Figure 1B, and both hydrogen-bonding distances given in this table are depicted by dashed lines. The species labels refer to the protonation states of A-1(I’O2’), G40(N1) and the position of the Mg\textsuperscript{2+} ion at the cleavage site. Data for the systems without an active site Mg\textsuperscript{2+} ion are reanalyzed from ref 20. Distances are given in Å. The numbers in parentheses are the standard deviations. Similar hydrogen-bonding patterns were determined from QM/MM geometry optimizations, as given in Table S1. The number before the slash is the Mg\textsuperscript{2+}:pro-S\textsubscript{P} distance, and the number after the slash is the Mg\textsuperscript{2+}:pro-R\textsubscript{P} distance. Average distances for only the frames that have the A-1(I’O2’):G40(N1) hydrogen bond. The free energy barrier associated with the rotation from the A-1(I’O2’):pro-R\textsubscript{P} hydrogen bond to the A-1(I’O2’):G40(N1) hydrogen bond is \(~1\) kcal/mol according to ref 20. dN1 Mg\textsuperscript{2+}@S\textsubscript{P} is not given because the Mg\textsuperscript{2+} moves to the S\textsubscript{P} position even when started at the R\textsubscript{P} position.”
Table 2. Average Distances from Classical MD Simulations with a Mg$^{2+}$ Ion near the Hoogsteen Face of G40$^{a}$

| species                  | Mg$^{2+}$ position $^b$ | GlcN6P(O1):pro-R<sub>p</sub> | A-1(O2'):G40(N1) |
|--------------------------|-------------------------|-------------------------------|------------------|
| canonical Mg$^{2+}$@O6   | 2.08 (0.06)/4.57 (0.19) | 2.83 (0.09)                   | 3.93 (0.42)      |
| canonical Mg$^{2+}$@N7   | 3.96 (0.12)/2.28 (0.17) | 2.72 (0.07)                   | 3.92 (0.29)      |
| dO2' Mg$^{2+}$@O6        | 2.34 (0.26)/4.33 (0.30) | 3.04 (0.08)                   | 4.93 (0.11)      |
| dN1Mg$^{2+}$@O6          | 1.98 (0.04)/3.94 (0.26) | 2.62 (0.08)                   | 3.93 (0.26)      |

“*The site at the Hoogsteen face of G40 is labeled as Site 2 in Figure 1B. The species labels refer to the protonation states of A-1(O2') and G40(N1) and the position of the Mg$^{2+}$ ion at the Hoogsteen face of G40. Distances are given in Å. The numbers in parentheses are the standard deviations. Similar hydrogen-bonding patterns were determined from QM/MM geometry optimizations, as given in Table S2, except that some of these hydrogen bonds were retained in the absence of conformational sampling. The number before the slash is the Mg$^{2+}$:G40(O6) distance, and the number after the slash is the Mg$^{2+}$:G40(N7) distance.

interaction of A-1(O2') with G40 was weakened, resulting in the movement of G40 away from the cleavage site. In addition, the protonated cofactor moved away from the active site, presumably due to electrostatic repulsion with the Mg$^{2+}$ ion. Table 1 provides average hydrogen-bonding distances from the classical MD simulations. Note that the hydrogen bonds between the cofactor and the pro-R<sub>p</sub> oxygen and between A-1(O2') and G40(N1) have been weakened or disrupted in the presence of a Mg$^{2+}$ ion at the cleavage site. QM/MM geometry optimizations confirmed the same type of disruption of the hydrogen-bonding interactions within the active site (Figure 2 and Table S1). Moreover, the O2'-P-O5' angle decreases from 172° without an active site Mg$^{2+}$ ion to 113° with a Mg$^{2+}$ ion near the pro-R<sub>p</sub> oxygen for the canonical state, indicating disruption of the in-line attack conformation.

In classical MD simulations of the dO2' state, the Mg$^{2+}$ ion is always stably coordinated to the deprotonated A-1(O2'). This coordination of the Mg$^{2+}$ ion to A-1(O2') will hinder the subsequent nucleophilic attack. A similar obstructive effect was also observed in the QM/MM free energy simulations for the full cleavage reaction starting with the dO2' state. For example, the calculated free energy barrier for the cleavage reaction with a Mg$^{2+}$ ion near the pro-S<sub>p</sub> oxygen for the dO2' state is ~31 kcal/mol (Figure S5), which is ~12 kcal/mol higher than the free energy barrier obtained from the same type of simulation for the analogous reaction pathway without the active site Mg$^{2+}$ ion. Note that the Mg$^{2+}$ ion is not coordinated to A-1(O2') at the beginning of the reaction pathway but becomes closer to this nucleophile as the reaction progresses (Figure SSD). This substantially higher free energy barrier obtained when a Mg$^{2+}$ ion is at the cleavage site is inconsistent with experimental measurements.

Overall, these collective simulation data indicate that the presence of a Mg$^{2+}$ ion at the cleavage site has a deleterious or anticatalytic effect on the self-cleavage mechanism. The first anticatalytic effect is that this Mg$^{2+}$ ion pushes the cofactor out of the active site due to electrostatic repulsion and disruption of the hydrogen bond between the cofactor and the pro-R<sub>p</sub> oxygen (Table 1). The second anticatalytic effect is that this Mg$^{2+}$ ion coordinates to A-1(O2') and therefore obstructs the nucleophilic attack (Figure S4B). Moreover, the free energy barrier for the cleavage reaction when a Mg$^{2+}$ ion is at the cleavage site is inconsistent with experimental data. Thus, these simulations suggest that a Mg$^{2+}$ ion is unlikely to be positioned at the cleavage site.

To explore another potential mechanistic role of the Mg$^{2+}$ ion, we investigated a second scenario, in which the Mg$^{2+}$ ion interacts with the Hoogsteen face of G40 (labeled “Site 2” in Figure 1B). The G40(N7) and G40(O6) shown in Figure 1B belong to the Hoogsteen face of the guanine and are able to coordinate to a Mg$^{2+}$ ion. Although no glmS ribozyme crystal structure with a Mg$^{2+}$ ion near the Hoogsteen face of G40 has been reported, the crystal structure of a glmS aporibozyme triple mutant exhibits a Ca$^{2+}$ ion near O6 of the Hoogsteen face of an adjacent guanine site with space for it to move to the Hoogsteen face of the putative base. Moreover, experimental mutation of G40 to U was found to decrease the self-cleavage rate by 3–5 orders of magnitude, and experiments performed in the presence of a 7-deaza-guanine substitution and Mn$^{2+}$ led to rate reduction that suggests the possibility of metal ion binding to the Hoogsteen face of G40. In general, metal ions are able to interact via various types of coordination with the O6 or the N7 of the Hoogsteen face of a guanine and lower the pK<sub>a</sub> of the NH of G. On the basis of this literature, it is not clear whether a Mg$^{2+}$ ion would interact more strongly with G40(O6) or G40(N7) in the glmS ribozyme environment. A classical free energy simulation performed with the string method combined with umbrella sampling indicated that the free energy difference between these two equilibrium configurations is ~1 kcal/mol, slightly

DOI: 10.1021/acs.jpcl.6b01854
focusing the G40(O6) position (Figure S6). Therefore, we explored two representative cases for this scenario: the first with a Mg2+ ion near G40(O6) and the second with a Mg2+ ion near G40(N7).

In contrast to the first scenario, in which the active site Mg2+ ion was at the cleavage site, the presence of a Mg2+ ion near the Hoogsteen face of G40 (Site 2 in Figure 1B) did not significantly disrupt the hydrogen-bonding pattern or the in-line attack conformation in the active site. Thus, unlike Site 1, Site 2 is not overtly anticatalytic. In these classical MD simulations, a Mg2+ ion was initially positioned at either G40(N7) or G40(O6). In the dN1 and dO2′ states, the metal ion moved closer to G40(O6), which is closer to the deprotonated moiety, for the trajectories in which Mg2+ was initially positioned at G40(N7). This movement was most likely due to electrostatic attraction between the Mg2+ ion and the deprotonated G40(N1) or A-1(O2′), although it did not move enough to coordinate directly to G40(N1) or A-1(O2′).

In the canonical state, the Mg2+ ion remained near its initial position. When the metal ion was near G40(N7), it also interacted with a nearby phosphate group from G40.

Table 2 provides the average distances and angles from these classical MD simulations. For all of these MD trajectories, the cofactor remained stably bound in the active site and formed a hydrogen bond with the pro-Rp oxygen. Compared to the MD simulations without an active site Mg2+ ion (data given in Table 1), the A-1(O2′):G40(N1) hydrogen bond became weaker in the presence of a Mg2+ ion near the Hoogsteen face of G40. Weakening of the hydrogen-bonding interaction between A-1(O2′) and G40(N1) is expected to lower the pKa of G40(N1) in the canonical state and therefore facilitate the initial deprotonation of G40(N1) by an external base, which is proposed to initiate the self-cleavage reaction. Thus, the presence of a Mg2+ ion at the Hoogsteen face of G40 could potentially be catalytically favorable. Although the A-1(O2′):G40(N1) hydrogen bond also becomes weaker when a Mg2+ ion is at the cleavage site (Site 1 in Figure 1B and Table 1), the accompanying weakening of the hydrogen bond between the cofactor and the pro-Rp oxygen, as well as the electrostatic repulsion between the Mg2+ ion and the cofactor, negates the potentially favorable effect in that case.

These issues were further explored by QM/MM geometry optimizations and pKa calculations with a Mg2+ ion at the Hoogsteen face of G40. QM/MM geometry optimizations confirmed the retention of most hydrogen-bonding interactions within the active site, as well as the weakening of the A-1(O2′):G40(N1) hydrogen bond upon placement of the Mg2+ ion at the Hoogsteen face (Figure 3 and Table S2). Furthermore, pKa calculations confirmed a decrease of the pKa value of G40(N1) when a Mg2+ ion is near the Hoogsteen face of G40. The calculated pKa of G40(N1) is 11.6 ± 1.5 without an active site Mg2+ ion and 7.9 ± 1.1 with a Mg2+ ion at the Hoogsteen face, predicting a decrease of 3.7 pKa units. This pKa shift is similar to the shifts measured experimentally for coordination of various metal ions to the N7 of G, which range from 1.4 to 2.5. In these pKa calculations, the MD configurations were selected to retain the A-1(O2′):G40(N1) hydrogen bond, where the hydrogen bond donor is either A-1(O2′) or G40(N1). Note that these error bars were determined from statistical analysis and do not account for systematic errors, but the relative pKa values are expected to be reliable.

This work has elucidated the potential anticatalytic and catalytic effects of an active site Mg2+ ion in the GlcN6P-bound glmS ribozyme. In general, Mg2+ ions have been shown to stabilize the scissile phosphate negative charge that develops during the cleavage reaction as well as to shift the pKa’s of active site residues. The simulations presented herein indicate that the glmS ribozyme does not employ a Mg2+ ion at the cleavage site (Site 1) because of two anticatalytic effects: (1) pushing the positively charged cofactor from the active site, presumably due to electrostatic repulsion, with concomitant disruption of the hydrogen bond between GlcN6P(O1) and the pro-Rp oxygen on the scissile phosphate, and (2) obstructing the nucleophile attack by A-1(O2′) on the scissile phosphate by coordination to the deprotonated A-1(O2′). The disruption of the hydrogen-bonding interactions within the active site also disfavors the presumed in-line attack conformation required for catalysis. These findings are consistent with studies from the Breaker lab as well as recent studies from our lab that support the absence of a metal ion at the active site of the holoribozyme. The calculations discussed herein suggest that the presence of a Mg2+ ion at the cleavage site (Site 1) in the holoribozyme is disruptive and therefore exerts an anticatalytic effect.

In contrast, the presence of a Mg2+ ion near the Hoogsteen face of G40 (Site 2) does not significantly disrupt the hydrogen-bonding interactions within the active site and does not exhibit any of the other anticatalytic effects. A possible catalytically favorable effect of the Mg2+ ion near the Hoogsteen face of G40 is the lowering of the pKa of G40(N1), thereby facilitating the initial deprotonation presumed to be required for the self-cleavage reaction. Further experimental and theoretical studies are required to fully unravel the diverse roles of Mg2+ ions in ribozymes.
AUTHOR INFORMATION

Corresponding Author
*E-mail: shs3@illinois.edu.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is funded by U.S. National Institutes of Health Grant GM056207 (S.H.-S.) and U.S. National Science Foundation Grant CHE-1213667 (P.C.B.). The free energy simulations were performed using allocations on STAMPEDE in the XSEDE supercluster groups.

REFERENCES

(1) Fedor, M. J.; Williamson, J. R. The Catalytic Diversity of RNAs. Nat. Rev. Mol. Cell Biol. 2005, 6, 399–412.
(2) Ferré-D’Amare, A. R.; Scott, W. G. Small Self-Cleaving Ribozymes. Cold Spring Harbor Perspect. Biol. 2010, 2, a003574.
(3) Wedekind, J. E. Metal Ion Binding and Function in Natural and Artificial Small RNA Enzymes from a Structural Perspective. Structural and Catalytic Roles of Metal Ions in RNA; Metal Ions Life Sciences; Royal Society of Chemistry: Cambridge, U.K., 2011; Vol. 9; pp 299–345. DOI: 10.1039/9781849732512-00299
(4) Shan, S. O.; Yoshida, A.; Sun, S. G.; Piccirilli, J. A.; Herschlag, D. Three Metal Ions at the Active Site of the Tetrahymena Group I Ribozyme. Proc. Natl. Acad. Sci. U. S. A. 1999, 96, 12299–12304.
(5) Pyle, A. M.; Sigel, R. K. O.; Vaidya, A. Metal Ion Binding Sites in a Group II Intron Core. Nat. Struct. Biol. 2000, 7, 1111–1116.
(6) Stahley, M. R.; Strobel, S. A. Structural Evidence for a Two-Metalion Mechanism of Group I Intron Splicing. Science 2005, 309, 1587–1590.
(7) Toor, N.; Keating, K. S.; Taylor, S. D.; Pyle, A. M. Crystal Structure of a Self-Spliced Group II Intron. Science 2008, 320, 77–82.
(8) Chen, J.; Ganguly, A.; Miswan, Z.; Hammes-Schiffer, S.; Bevilacqua, P. C.; Golden, B. L. Identification of the Catalytic Mg–Ion in the Hepatitis Delta Virus Ribozyme. Biochemistry 2013, 52, 557–567.
(9) McCarthy, T. J.; Plog, M. A.; Floy, S. A.; Jansen, J. A.; Soukup, J. K.; Soukup, G. A. Ligand Requirements for glmS Ribozyme Self-Cleavage. Chem. Biol. 2005, 12, 1221–1226.
(10) Klein, D. J.; Ferré-D’Amare, A. R. Structural Basis of glmS Ribozyme Activation by Glucosamine-6-Phosphate. Science 2006, 313, 1752–1756.
(11) Cochrane, J. C.; Lipchock, S. V.; Strobel, S. A. Structural Investigation of the glmS Ribozyme Bound to Its Catalytic Cofactor. Chem. Biol. 2007, 14, 97–105.
(12) Klein, D. J.; Been, M. D.; Ferré-D’Amare, A. R. Essential Role of an Active-Site Guanine in glmS Ribozyme Catalysis. J. Am. Chem. Soc. 2007, 129, 14858–14859.
(13) Cochrane, J. C.; Lipchock, S. V.; Smith, K. D.; Strobel, S. A. Structural and Chemical Basis for Glucosamine-6-Phosphate Binding and Activation of the glmS Ribozyme. Biochemistry 2009, 48, 3239–3246.
(14) Banas, P.; Walter, N. G.; Sponer, J.; Otyepka, M. Protonation States of the Key Active Site Residues and Structural Dynamics of the glmS Riboswitch as Revealed by Molecular Dynamics. J. Phys. Chem. B 2010, 114, 8701–8712.
(15) Xin, Y.; Hamelberg, D. Deciphering the Role of Glucosamine-6-Phosphate in the Riboswitch Action of glmS Ribozyme. RNA 2010, 16, 2455–2463.
(16) Gong, B.; Klein, D. J.; Ferré-D’Amare, A. R.; Carey, P. R. The glmS Ribozyme Tunes the Catalytically Critical pKₐ of Its Coenzyme Glucosamine-6-phosphate. J. Am. Chem. Soc. 2011, 133, 14188–14191.
(17) Viladoms, J.; Scott, L. G.; Fedor, M. J. An Active-Site Guanine Participates in glmS Ribozyme Catalysis in Its Protonated State. J. Am. Chem. Soc. 2011, 133, 18388–18396.
(18) Viladoms, J.; Fedor, M. J. The glmS Ribozyme Cofactor is a General Acid-Base Catalyst. J. Am. Chem. Soc. 2012, 134, 19043–19049.
(19) Dubeyck, M.; Walter, N. G.; Sponer, J.; Otyepka, M.; Banas, P. Chemical Feasibility of the General Acid/Base Mechanism of glmS Ribozyme Self-Cleavage. Biopolymers 2015, 103, 550–562.
(20) Zhang, S.; Ganguly, A.; Goyal, P.; Bingaman, J. L.; Bevilacqua, P. C.; Hammes-Schiffer, S. Role of the Active Site Guanine in the glmS Ribozyme Self-Cleavage Mechanism: Quantum Mechanical/Molecular Mechanical Free Energy Simulations. J. Am. Chem. Soc. 2015, 137, 784–798.
(21) Klawuhn, K.; Jansen, J. A.; Souchek, J.; Soukup, G. A.; Soukup, J. K. Analysis of Metal Ion Dependence in glmS Ribozyme Self-Cleavage and Coenzyme Binding. ChemBioChem 2010, 11, 2567–2571.
(22) Brooks, K. M.; Hampel, K. J. Rapid Steps in the glmS Ribozyme Catalytic Pathway: Cation and Ligand Requirements. Biochemistry 2011, 50, 2424–2433.
(23) Klein, D. J.; Wilkinson, S. R.; Been, M. D.; Ferré-D’Amare, A. R. Requirement of Helix p2.2 and Nucleotide G1 for Positioning the Cleavage Site and Cofactor of the glmS Ribozyme. J. Mol. Biol. 2007, 373, 178–189.
(24) Rosta, E.; Nowotny, M.; Yang, W.; Hammer, G. Catalytic Mechanism of RNA Backbone Cleavage by Ribonuclelease H from Quantum Mechanics/Molecular Mechanics Simulations. J. Am. Chem. Soc. 2011, 133, 8934–8941.
(25) Gilson, M. K.; Honig, B. H. The Dielectric-Constant of a Folded Protein. Biopolymers 1986, 25, 2097–2119.
(26) Archontis, G.; Simpson, T. Proton Binding to Proteins: A Free-Energy Component Analysis Using a Dielectric Continuum Model. Biophys. J. 2005, 88, 3908–3904.
(27) Bingaman, J. L.; Zhang, S.; Stevens, D. R.; Yennawar, N. H.; Hammes-Schiffer, S.; Bevilacqua, P. C. GlcN6P Cofactor Serves Multiple Catalytic Roles in the glmS Ribozyme. Submitted.
(28) Mir, A.; Golden, B. L. Two Active Site Divalent Ions in the Crystal Structure of the Hammerhead Ribozyme Bound to a Transition State Analogue. Biochemistry 2016, 55, 633–636.
(29) Lau, M. W. L.; Ferré-D’Amare, A. R. An in vitro Evolved glmS Ribozyme Has the Wild-Type Fold but Loses Coenzyme Dependence. Nat. Chem. Biol. 2013, 9, 805–810.
(30) Egli, M.; Gessner, R. V.; Williams, L. D.; Quigley, G. J.; Vandermarel, G. A.; Vanboom, J. H.; Rich, A.; Frederick, C. A. Atomic-Resolution Structure of the Cellulose Synthase Rotor Cyclic Diacylglycerol Acid. Proc. Natl. Acad. Sci. U. S. A. 1990, 87, 3235–3239.
(31) Juneau, K.; Podell, E.; Harrington, D. J.; Cech, T. R. Structural basis of the enhanced stability of a mutant ribozyme domain and a detailed view of RNA-solvent interactions. Structure 2001, 9, 221–231.
(32) Freisinger, E.; Sigel, R. K. O. From Nucleotides to Ribozymes - A Comparison of Their Metal Ion Binding Properties. Coord. Chem. Rev. 2007, 251, 1834–1851.
(33) Sigel, R. K. O.; Sigel, H. Comprehensive Inorganic Chemistry II, 2nd ed; Elsevier Ltd: Amsterdam, The Netherlands, 2013; pp 623–660, Chapter 3.21.
(34) Leonarski, F.; D’Ascenzo, L.; Aufinger, P. Binding of Metals to Purine N7 Nitrogen Atoms and Implications for Nucleic Acids: A CSD Survey. Inorg. Chem. Acta 2016, DOI: 10.1016/j.inorgchem.2016.04.005.
(35) Roth, A.; Nahvi, A.; Lee, M.; Jona, I.; Breaker, R. R. Characteristics of the glmS Ribozyme Suggest Only Structural Roles For Divalent Metal Ions. RNA 2006, 12, 607–619.