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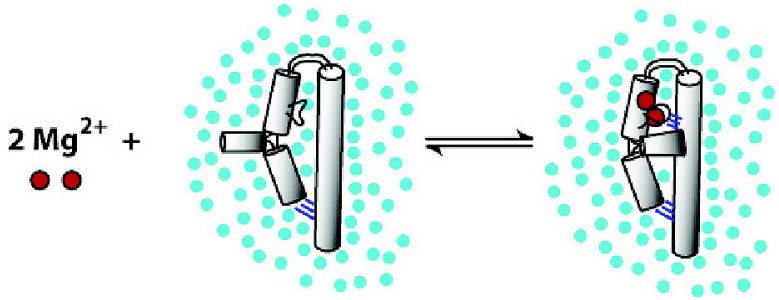

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Determining the Mg^{2+} Stoichiometry for Folding an RNA Metal Ion Core

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In living organisms and in test tubes, structured RNA molecules make intimate interactions with divalent metal cations, especially magnesium. Numerous thermodynamic, structural, and biochemical studies have probed important aspects of metal ion association with nucleic acids,[1,2] and there is a rich literature on the theory of ion−polyelectrolyte interactions.[3] Nevertheless, a basic question remains unanswered for RNAs studied to date: how many Mg^{2+} ions are involved in an RNA folding event?

One widely used approach to estimate metal ion stoichiometry for RNA folding events has relied on fitting equilibrium data to the Hill equation (eq 1).[2,4] This approach implicitly assumes full cooperativity of folding, in which case the apparent Hill coefficient (n_{Hill}) for Mg^{2+} dependence of the folding equilibrium constant (K_{obs}) equals the Mg^{2+} ion uptake upon folding ($n_{fold} - n_{unfold}$).[5]

$$\frac{\partial \log K_{obs}}{\partial \log [Mg^{2+}]} = n_{Hill}; \quad n_{Hill} = n_{fold} - n_{unfold} \qquad (1)$$

There are, however, fundamental problems in interpreting the Hill coefficient as the number of Mg^{2+} ions bound to specific sites upon folding of an RNA. First, the Mg^{2+} ions of the diffuse ion "atmosphere" that surrounds the RNA backbone can contribute substantially to the Hill coefficient. Thus, the Hill coefficient need not reflect the number of site-bound Mg^{2+} ions.[2] Further, the shape and the ion composition of the atmosphere change as a function of ion concentration for both the unfolded and the folded states. As a result, a Hill coefficient measured near the Mg^{2+} midpoint may not correctly describe the stoichiometry at the higher Mg^{2+} concentrations typically used for functional and structural assays.[2] Finally, interpretation of the Hill equation and coefficient relies on the assumption of a cooperative two-state equilibrium. However, RNA molecules generally fold through multiple equilibrium intermediates,[6] and these multiple transitions are not straightforward to distinguish by standard experimental techniques, such as absorbance and gel mobility.[7]

Thus, Hill fits of RNA folding equilibrium data typically give incorrect estimates of Mg^{2+} association.[2] Therefore, it is necessary to directly count Mg^{2+} ions to determine the basic ion stoichiometry. In particular, the number of Mg^{2+} ions associated with an RNA's unfolded and folded states (n_{unfold} and n_{fold}, respectively) must be measured. If, under some conditions, the observed Mg^{2+} ion uptake does equal the Hill coefficient (eq 1), strong support is provided for the two-state behavior assumed in the Hill analysis and for a constant stoichiometry of metal ion association across the entire folding transition. Testing these assumptions is a prerequisite for rigorous thermodynamic interpretation of metal ion interactions with structured RNAs.

We demonstrate herein that the Hill relation holds for the folding of an RNA "metal ion core" observed in the crystal structure of the 158 nucleotide P4−P6 domain of the *Tetrahymena* group I ribozyme[8,10] (Figure 1a) in 2 M NaCl. The high monovalent salt competes away divalent ions from the ion atmosphere and thereby helps to isolate energetic and spectroscopic signatures of any site-bound divalent ions.[11,12] Upon reducing the large, varying atmospheric contribution, the Hill coefficient for Mg^{2+} observed near the folding midpoint is expected to be applicable over a wide range of Mg^{2+} concentrations and to put a strong limit on the number of specific Mg^{2+} binding sites (eq 1).[11] Furthermore, the suppression of the atmospheric Mg^{2+} background lowers the magnitudes of n_{unfold} and n_{fold} so that their difference can be measured with sufficient precision to allow a meaningful comparison to the Hill coefficient.

In a background of 2 M NaCl, the P4−P6 RNA exhibits a compact structure with a hydroxyl radical reactivity profile identical to that of the native state for all backbone residues except those that form the P5abc "magnesium ion core" and the core's P4 helix docking site.[8,10,13] Addition of $MgCl_2$ induces the folding of these core residues (Figure 1a), leading to a radical reactivity profile for the entire molecule that is indistinguishable from the native state. The protections of residues throughout the metal ion core region display indistinguishable dependences on Mg^{2+} concentration, with a midpoint of 0.52 ± 0.03 mM and an apparent Hill coefficient of 1.8 ± 0.1 (Figure 1b), consistent with a cooperative, two-state process.[14] The Hill analysis thus suggests that folding the P4−P6 metal ion core requires only two Mg^{2+} ions, if the assumptions of constant stoichiometry and two-state folding are correct.

The assumptions inherent to the Hill analysis were directly tested by measuring the difference between n_{fold} and n_{unfold}. Two independent techniques were employed to measure the small numbers of ions associated with metal ion core folding and gave the same values within error. The dye 8-hydroxyquinoline-5-sulfonic acid (HQS) offers a fluorescent readout of free Mg^{2+} concentration that can be subtracted from the known total Mg^{2+} concentration added to an RNA sample to give the number of Mg^{2+} ions associated with the RNA.[7] Equilibrium dialysis of the RNA sample to a known free Mg^{2+} concentration, followed by measurement of total Mg^{2+} concentration by atomic emission spectroscopy (AES), provides an independent count of the associated Mg^{2+} ions.[15]

The number of Mg^{2+} ions associated with the wild-type P4−P6 RNA deviates markedly from control measurements for a mutant that shows no Mg^{2+}-dependent metal ion core protections[13] (Figure 2a). This deviation occurs over the concentration range for metal ion core folding (0.2−1 mM Mg^{2+}, Figure 1b); above the folding transition, the number of associated Mg^{2+} ions increases in parallel for the folded and mutant RNAs. After subtracting the mutant from the wild-type values, a Mg^{2+} association curve is observed with a midpoint and steepness consistent with the footprinting data (Figure 2b). The value of this curve well above the folding midpoint gives the total uptake of Mg^{2+} ions upon metal ion core folding; the limiting value of 1.9 ± 0.2 ions (HQS) or 1.9 ± 0.1 ions (AES) is

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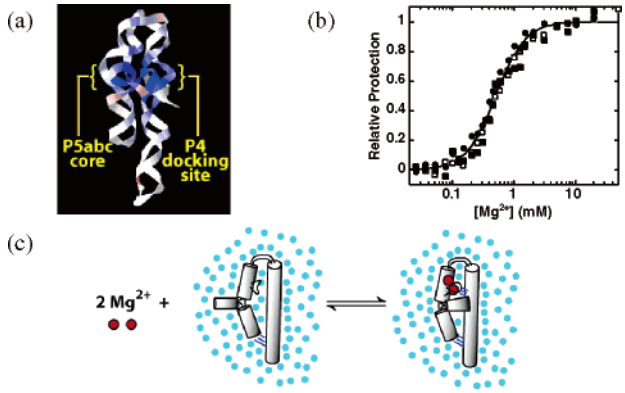

Figure 1. Folding of the P4−P6 RNA metal ion core. (a) Difference in RNA hydroxyl radical cleavage pattern in the presence and absence of 10 mM $MgCl_2$, in a background of 2 M NaCl; deep blue represents protections in Mg^{2+} of 30% or more.[8,13,14] (b) Mg^{2+}-dependent protection (linearly scaled to increase from 0 to 1) of representative residues in the metal ion binding core (●, 182; and ■, 185) and in the core's docking site (□, 214) fit the Hill relation with midpoint 0.52 mM and apparent Hill coefficient 1.8 (solid line). Absolute protection values and additional probed residues are given in Supporting Information. (c) Schematic representation of the folding equilibrium for the P4−P6 RNA upon Mg^{2+} addition in a high monovalent ion background. The folding occurs with association of two Mg^{2+} ions (red; depicted as site-bound in this model), accompanied by a loss of Na^+ ions (cyan) and/or uptake of Cl^- ions (not shown) to maintain charge neutrality.[2,9]

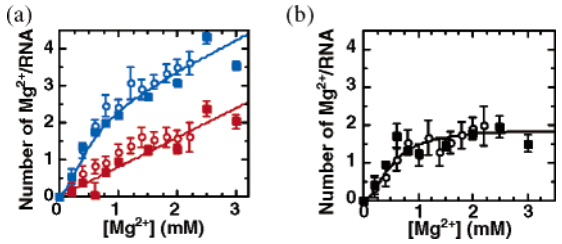

Figure 2. Counting the number of Mg^{2+} ions associated with the folding of the P4−P6 RNA metal ion core through the use of a Mg^{2+} indicator dye (open symbols) and atomic emission spectroscopy (closed symbols), in the presence of 2 M NaCl. For practical reasons, indicator data were obtained only to 2.2 mM Mg^{2+}. (a) Measurements of Mg^{2+} ions associated with the wild-type RNA (blue) and with a mutant (red) that does not form a structured metal ion core.[13] Solid lines show a simple linear fit for nonspecific Mg^{2+} association to the mutant (red) and a fit to the wild-type (blue) that includes a Hill contribution (midpoint, 0.52 mM, from Figure 1b; and $n_{Hill} = 1.9$ fit to these data) for Mg^{2+} uptake upon metal ion core folding. (b) Differences between measurements for the wild-type and mutant agree with a Hill curve (solid line) with a midpoint of 0.52 mM (from Figure 1b) and a fitted uptake of 1.9 Mg^{2+} ions upon metal ion core folding.

in close agreement with the Hill coefficient of 1.8 ± 0.1 obtained by footprinting, thereby validating the Hill analysis (eq 1).

Our stoichiometric measurements show that two metal ions induce folding of the P4−P6 core in a two-state manner throughout the Mg^{2+} titration.[16] Future titrations with other divalent metal ions[12,17] and with specific P4−P6 mutants[10] will provide a complete portrait of the binding modes of these two Mg^{2+} ions (diffuse or specific; see below) and their energetic connectivity. The knowledge that two ions are captured upon metal ion core folding will allow such titrations to be interpreted with a thermodynamic rigor unprecedented for RNA folding.

The two-metal-ion stoichiometry demonstrated herein appears to contradict the observation of five Mg^{2+} ions with at least one direct contact with an RNA ligand in P4−P6 crystal structures.[8,10,18] Our data do not pinpoint the locations of the two Mg^{2+} ions captured in our high monovalent salt solution conditions, and it is even possible that one or both ions are diffusely associated with the RNA,[19] in further contrast to the crystal structure. We speculate, however, that the two Mg^{2+} ions are indeed bound to the two crystallographic sites that involve three direct contacts to the tightly turned and deeply buried A-rich bulge, while the other crystallographic Mg^{2+} ions are replaced by Na^+ ions, either diffusely or specifically bound. Phosphorothioate substitution and rescue experiments,[10] with titrations of soft divalent metal ions monitored by footprinting and spectroscopy,[20] may provide tests of this structural model.

We expect the combination of Hill analysis and ion counting technologies presented herein to be a powerful method for illuminating the metal ion stoichiometries of RNAs that require Mg^{2+} for folding and catalysis. Attaining such precise descriptions of RNA/metal ion interactions under high monovalent salt conditions further provides a necessary foundation for dissecting the more complex phenomenology of RNA behavior at lower, physiological ionic strengths at which Mg^{2+} ions dominate the atmosphere and multiple RNA conformational transitions occur.

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Supporting Information Available: Descriptions of hydroxyl radical footprinting, AES, and HQS techniques; radical cleavage profiles for RNA states discussed herein; Hill fits for individual residues; AES with control DNA duplexes; and tests of alternative models with more than two metal ions in the core. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Pan, T.; Long, D. M.; Uhlenbeck, O. C. In *The RNA World*; Gesteland, R. F., Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1993; pp 271−302. (b) Record, M. T., Jr.; Zhang, W.; Anderson, C. F. *Adv. Protein Chem.* **1998**, *51*, 281−353.
(2) Draper, D. E. *RNA* **2004**, *10*, 335−43.
(3) Grosberg, A. Y.; Nguyen, T. T.; Shklovskii, B. I. *Rev. Mod. Phys.* **2002**, *74*, 329.
(4) Hill, A. V. *J. Physiol.* **1910**, *40*, iv.
(5) A small contribution to the observed Mg^{2+} Hill coefficient due to uptake of negative chloride co-ions upon folding can be neglected in our working conditions, as the added chloride concentration (<10 mM) is much less than the background concentration (2 M).
(6) (a) Celander, D. W.; Cech, T. R. *Science* **1991**, *251*, 401−7. (b) Fang, X.; Pan, T.; Sosnick, T. R. *Biochemistry* **1999**, *38*, 16840−6. (c) Shelton, V. M.; Sosnick, T. R.; Pan, T. *Biochemistry* **2001**, *40*, 3629−38.
(7) Romer, R.; Hach, R. *Eur. J. Biochem.* **1975**, *55*, 271−84.
(8) Cate, J. H.; Gooding, A. R.; Podell, E.; Zhou, K.; Golden, B. L.; Kundrot, C. E.; Cech, T. R.; Doudna, J. A. *Science* **1996**, *273*, 1678−85.
(9) Uchida, T.; He, Q.; Ralston, C. Y.; Brenowitz, M.; Chance, M. R. *Biochemistry* **2002**, *41*, 5799−806.
(10) Cate, J. H.; Hanna, R. L.; Doudna, J. A. *Nat. Struct. Biol.* **1997**, *4*, 553−8.
(11) (a) Leroy, J. L.; Gueron, M. *Biopolymers* **1977**, *16*, 2429−46. (b) Horton, T. E.; Clardy, D. R.; DeRose, V. J. *Biochemistry* **1998**, *37*, 18094−101. (c) Wilson, T. J.; Lilley, D. M. *RNA* **2002**, *8*, 587−600.
(12) Bukhman, Y. V.; Draper, D. E. *J. Mol. Biol.* **1997**, *273*, 1020−31.
(13) Takamoto, K.; Das, R.; He, Q.; Doniach, S.; Brenowitz, M.; Herschlag, D.; Chance, M. R. *J. Mol. Biol.* **2004**, *343*, 1195−206.
(14) An analogous experiment with a 2 M KCl background gives a similar Hill coefficient of 2.0 ± 0.1 and a slightly lower Mg^{2+} midpoint, 0.37 ± 0.03 mM (Supporting Information).
(15) Bina-Stein, M.; Stein, A. *Biochemistry* **1976**, *15*, 3912−7.
(16) Only three Mg^{2+} ions are associated with wild-type P4−P6 above the folding transition (1.2 mM Mg^{2+}), eliminating all models with more than three Mg^{2+} ions bound to the metal ion core. Models with the three associated Mg^{2+} ions that are all core-bound give poor fits to the wild-type data (Supporting Information). Further, the association of one Mg^{2+} ion with mutant P4−P6 and with a duplex control strongly suggests that at least one of the three metal ions is nonspecifically associated.
(17) Rangan, P.; Woodson, S. A. *J. Mol. Biol.* **2003**, *329*, 229−38.
(18) Juneau, K.; Podell, E.; Harrington, D. J.; Cech, T. R. *Structure* **2001**, *9*, 221−31.
(19) Misra, V. K.; Draper, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12456−61.
(20) Maderia, M.; Hunsicker, L. M.; DeRose, V. J. *Biochemistry* **2000**, *39*, 12113−20.

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