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Differential Binding of Mg^{2+}, Zn^{2+}, and Cd^{2+} at Two Sites in a Hammerhead Ribozyme Motif, Determined by ^{15}N NMR

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The small, well-characterized hammerhead ribozyme (Figure 1) has served as a useful model for understanding the catalytic activity of RNA for the last two decades.[1] A wealth of biochemical data[2] was brought into focus by a series of X-ray crystallographic structures,[3] and a full picture of this remarkable molecule is still evolving as new information is discovered.[4] Divalent metal binding plays a crucial role in forming and maintaining key structures in all RNA, whether or not the metals participate in any catalytic steps.[5] Because the exact location of metals in solution can be difficult to identify with certainty, particularly the biologically ubiquitous Mg^{2+}, the full scope of metal binding to RNA is not yet defined. Most Mg^{2+} binds to RNA with its very tight hydration layer intact.[6] Binding of partially dehydrated Mg^{2+} only occurs when Mg^{2+} is directly chelated by two or more RNA ligands arrayed in a particularly favorable geometry, as has been observed in crystal structures of RNA involving a phosphate oxygen anion along with a uracil O4[6] or a guanine N7.[7] In contrast, the softer metals Cd^{2+} and Zn^{2+} are more easily dehydrated and thus have less stringent binding requirements.

^{15}N NMR is an ideal, nonperturbing method to probe directly for metals that are bound to nitrogen atoms in solution, since the ^{15}N chemical shift is very sensitive to changes in the local environment.[8] For example, a 20 ppm upfield change occurs for the N7 of guanosine in DMSO upon addition of 1 equiv of Zn^{2+} or Hg^{2+},[9] a ~70 ppm upfield change occurs for the N1 of adenosine upon protonation,[10,11] and smaller upfield changes (typically 2−9 ppm) occur upon formation of specific hydrogen bonds by purine N1 or N7 atoms in oligonucleotide duplexes and triplexes.[12]

The hammerhead ribozyme consists of three helices extending from a central core.[3] In the solid state, Domain II is known to contain a metal binding site adjacent to a tandem GA pair.[1] Although it is 20 Å from the cleavage site in Domain I, this metal binding site plays a vital role in catalysis.[13] In crystal structures, the A9 phosphate and the G10.1 N7 are both directly coordinated to a metal ion.[3] In solution, a self-complementary decamer that models this metal binding motif showed small, specific ^{31}P NMR chemical shift changes (0.3 ppm) for the binding site phosphate upon addition of Mg^{2+},[14] and small ^{1}H chemical shift changes (<1 ppm) for the binding site GH8 upon addition of $Co(NH_3)_6^{3+}$.[15] More strikingly, several versions of the same decamer with uniformly ^{13}C,^{15}N-labeled guanosine at different positions showed large upfield ^{15}N NMR chemical shift changes (~20 ppm) at the binding site GN7 upon addition of Cd^{2+}, with only 5−10 ppm changes at the other guanines.[16] Recently reported calculated ^{15}N NMR chemical shift values are in agreement with these data.[17]

As part of our long-standing interest in the use of ^{15}N NMR for exploring many kinds of nitrogen interactions in DNA and RNA,[11,12,18] we now report its value for comparing the binding of different metals to a given site, as well as comparing the binding of a given metal to different sites. We synthesized a nonself-complementary duplex (Figure 1) that models Domain II. Its sequence matches the core of the coaxially stacked Stems II and III, except for two base pairs at one end and without the three-strand junction. It contains [8-^{13}C-1,7,NH_2-$^{15}N_3$]-guanosine[19] at the expected binding site (G10.1) and [2-^{13}C-1,7,NH_2-$^{15}N_3$]-guanosine,[20] for comparison, at G16.2. The ^{13}C atoms serve as "tags" to allow differentiation of similar ^{15}N NMR signals[19,20] and also provide an additional, albeit indirect, probe for metal binding.

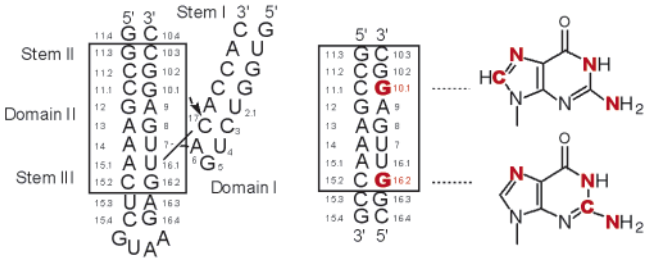

Figure 1. Structure of the hammerhead ribozyme (left), sequence of the decamer duplex (center) containing [8-^{13}C-1,7,NH_2-$^{15}N_3$]-guanosine at the binding site G10.1 and [2-^{13}C-1,7,NH_2-$^{15}N_3$]-guanosine at the nonbinding site G16.2, and structures of the labeled guanosines (right).

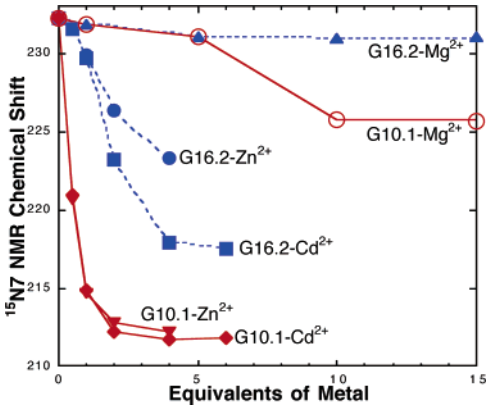

Figure 2. Plot of $^{15}N7$ chemical shifts vs equivalents of metal added.

We divided the RNA into identical samples, each 3.0 mM in duplex with 50 mM NaCl and 20 mM HEPES, and titrated them with the diamagnetic metals, Mg^{2+}, Cd^{2+}, or Zn^{2+}, while monitoring by ^{15}N and ^{13}C NMR at 20 °C. We observed large upfield ^{15}N chemical shift changes at G10.1 but not at G16.2. As shown in Figure 2, in the presence of 0.5 equiv (relative to the duplex) of either Zn^{2+} or Cd^{2+}, the G10.1 N7 signal moved upfield by ~11 ppm (and broadened significantly, more so with Cd^{2+} than Zn^{2+}). In contrast, that for G16.2 moved upfield by only 0.7 ppm and remained sharp. The presence of 1 equiv of either Zn^{2+} or Cd^{2+} caused a 17−18 ppm change for G10.1 and a sharpening of the signal (less so with Cd^{2+} than Zn^{2+}) but only a 2−3 ppm upfield change for G16.2. Thus, ^{15}N NMR clearly shows a very large and

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selective chemical shift change upon binding of one equivalent of Zn^{2+} or Cd^{2+} to G10.1. Further, the broadening at 0.5 equiv most likely reflects exchange between unbound and metal-bound states, with the subsequent sharpening indicative of the fully bound state. These upfield changes for G10.1 leveled off at 20 ppm with additional metal, with all signals sharp, while the upfield changes for G16.2 increased with additional metal, along with some broadening. We stopped the titrations after 4 equiv of Zn^{2+} and 6 equiv of Cd^{2+}, when the samples showed small amounts of precipitation.

In contrast to the relatively soft Zn^{2+} and Cd^{2+} ions, the harder Mg^{2+} normally does not bind to isolated nitrogens, including those in RNA. For example, we found that a sample of [7-^{15}N]-guanosine in the above buffer showed a 2.8 ppm upfield change with 10 equiv of Zn^{2+}, but only a 0.5 ppm upfield change with 100 equiv of Mg^{2+}, consistent with the ability of Zn^{2+}, but not Mg^{2+}, to interact to some extent with any basic nitrogen atom. Nevertheless, addition of 10 equiv of Mg^{2+} to the RNA duplex sample brought about a 6.5 ppm upfield shift for G10.1, along with considerable broadening, while G16.2 showed only a 1.2 ppm upfield change and only modest broadening. At 15 equiv of Mg^{2+} the sample showed a small amount of precipitation with no further chemical shift change for either N7.[21]

Thus, in our hammerhead motif, we see the same preference for binding at G10.1 over G16.2 for Mg^{2+}, Cd^{2+}, and Zn^{2+}, showing that experiments with softer metals, with less stringent binding criteria than Mg^{2+}, may nevertheless serve to identify potential Mg^{2+} binding sites. It is likely that Mg^{2+} is limited to binding by an electrostatic interaction with the G10.1 N7, while Cd^{2+} and Zn^{2+} can also bind covalently, perhaps accounting for the larger chemical shift changes observed with the latter metals. It is striking that this relatively small motif (a G adjacent to the A of a sheared GA pair) serves to create a binding pocket that is able to display such a strong, preferential interaction with metals. That the crystal structures of the hammerhead ribozyme show metals in precisely the same location is an example of the modular nature of RNA, which often combines independent domains into larger structures.

The other labeled guanine nitrogens in this duplex (N1s and aminos) displayed chemical shift changes no greater than 3 ppm with all metals, showing that, at least for this example, only the G10.1 N7 is a good ligand. ^{13}C NMR and ^{1}H NMR have also been used to monitor metal binding to a GN7, although the chemical shift changes are much smaller.[14,15] Similarly, we observed 1−2 ppm downfield changes of the ^{13}C8 in G10.1 during the titrations. In contrast, ^{15}N NMR is a far more sensitive method that gives much larger chemical shift changes and can be used directly to evaluate the binding of different metals, including Mg^{2+}, to a given nitrogen, as well as to compare the binding potential of different sites.

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Supporting Information Available: Experimental methods, partial ^{15}N NMR spectra, tables of ^{15}N NMR chemical shifts, and 2D HSQC spectrum. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) We added $Co(NH_3)_6^{3+}$ to a fourth sample and found very small (<1 ppm), identical downfield changes in the N7 chemical shift at both G10.1 and G16.2, with up to 6 equiv, at which point a small amount of precipitation appeared. Thus, there was no ^{15}N chemical shift evidence of binding for this metal.

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