Communication

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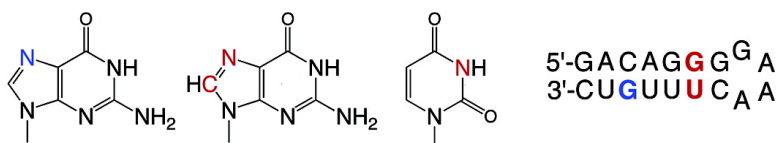
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RNA GG·UU Motif Binds K$^+$ but Not Mg^{2+}

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RNA requires metal ions to help form and maintain its active structure and also to carry out the wide range of functions it serves in all biological systems, including catalysis.[1] As RNA folds into its native structure, preformed[2] metal binding motifs that remain partially exposed may facilitate subsequent formation of more complex tertiary structure through additional interactions. Some preformed sites contain a guanine N7 along with one or two precisely positioned phosphates that chelate Mg^{2+} with high affinity, while others may contain no phosphates yet still participate in RNA folding through transient lower-affinity interactions. Although the biologically abundant yet spectroscopically silent ions Mg^{2+} and K$^+$ have been identified in crystal structures,[3–6] their specific binding has been difficult to detect in solution.

^{15}N NMR of ^{15}N-labeled nucleosides and nucleic acids is a nonperturbing method that is particularly sensitive to protonation, metal interactions, and hydrogen bonding.[7] For example, a ∼70 ppm upfield change occurs for the N1 of adenosine upon protonation,[8] a 20 ppm upfield change occurs for the N7 of guanosine upon addition of Zn^{2+} or Hg^{2+},[9] and smaller upfield changes of a few ppm occur upon formation of specific hydrogen bonds by purine N1 or N7 atoms in oligonucleotide duplexes and triplexes.[10]

We have recently reported the use of ^{15}N NMR to evaluate and compare the relative binding abilities of several metals to a ^{15}N specifically labeled RNA duplex designed to model a part of the hammerhead ribozyme.[11] The duplex contained a GA·AG motif that creates a high-affinity, preformed metal binding site between a specific phosphate and the N7 of an adjacent guanine.[12] We demonstrated that Mg^{2+}, Zn^{2+}, and Cd^{2+}, but not Co(NH$_3$)$_6$$^{3+}$, were specifically bound. We now report a comparison of metal binding at a very different kind of preformed metal binding motif, GG·UU pairs, in a hairpin that models the P5b stem loop of the self-splicing *Tetrahymena* group I intron.[13] We synthesized the same model hairpin that Tinoco[14] had studied several years ago, but with [8-^{13}C-7-^{15}N]-guanosine at G6 in the binding motif, [7-^{15}N]-guanosine at G16 outside the binding motif, for comparison, and [3-^{15}N]-uridine at U13 (Figure 1). The ^{13}C atom served primarily as a tag to differentiate unambiguously between the two ^{15}N7 NMR signals.[15]

We prepared six identical 3.3 mM samples of the labeled hairpin, each containing 50 mM NaCl and 20 mM HEPES, titrated them with Co(NH$_3$)$_6$$^{3+}$, Mg^{2+}, Zn^{2+}, Cd^{2+}, Na$^+$, and K$^+$, and monitored their ^{15}N resonances at 15 °C. As shown in Figure 2, we observed a 6.3 ppm *downfield* ^{15}N chemical shift change at the G6 N7 upon addition of 5 equiv of Co(NH$_3$)$_6$$^{3+}$.[16] The data show an apparent K_d of 520 μM ± 40. The signal displayed moderate broadening with 0.5 equiv, indicative of intermediate exchange between bound and unbound states, but sharpened with additional metal. At the G16 N7 we observed only a 1.8 ppm downfield change with 5 equiv and no broadening. This pronounced selective effect for Co(NH$_3$)$_6$$^{3+}$ is consistent with the binding to the GG·UU pairs that was seen in both crystal[13] and NMR[14] structures.
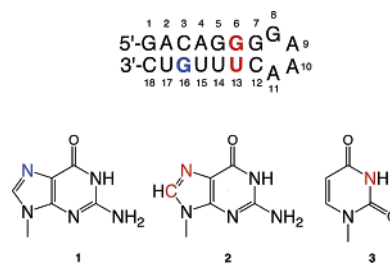
Figure 1. A labeled hairpin containing GG·UU pairs that models the P5b stem loop of the self-splicing *Tetrahymena* group I intron with [8-^{13}C-7-^{15}N]-guanosine (**2**) at G6 in the binding motif, [7-^{15}N]-guanosine (**1**) at G16 outside it, and [3-^{15}N]-uridine (**3**) at U13.
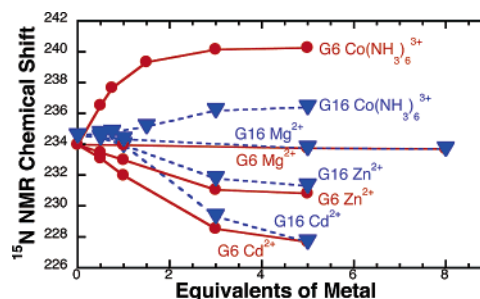
Figure 2. Plots of ^{15}N chemical shift for the labeled RNA hairpin as a function of added Co(NH$_3$)$_6$$^{3+}$, Mg^{2+}, Zn^{2+}, and Cd^{2+}.
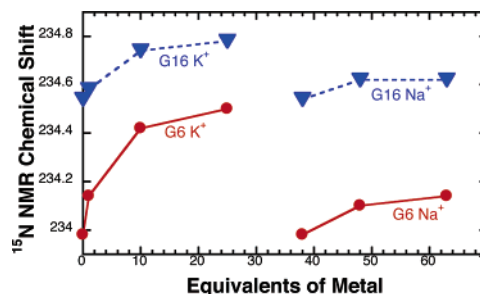
Figure 3. Plots of ^{15}N chemical shift for the labeled RNA hairpin as a function of added Na$^+$ and K$^+$.

We also found a *nonselective* upfield change of ∼5 ppm upon addition of 3 equiv of Cd^{2+}, a *nonselective* upfield change of ∼3 ppm upon addition of 3 equiv of Zn^{2+}, and a *nonselective* upfield change of < 1 ppm upon addition of 8 equiv of Mg^{2+}.[16] These results are entirely opposite to those we had seen for the GA·AG motif, to which Zn^{2+}, Cd^{2+}, and Mg^{2+} all bound strongly and selectively.[11]

Figure 3 shows the chemical shift changes caused by addition of Na$^+$ and K$^+$. The K$^+$ data give a curve consistent with selective binding to the GG·UU motif.[17] The apparent K_d of 6.3 mM ± 1.2 reflects a modest affinity for this interaction. The samples all contained 126 mM Na$^+$, so that we do not have data for small

amounts of Na^+, and see only a small linear downfield drift upon addition of more Na^+, consistent with the purely electrostatic interaction of diffuse ions.[18,19] The total chemical shift changes with these monovalent ions are of course significantly smaller than for the di- and trivalent ions.[20]

The ^{15}N chemical shift of a solvated N7 is known to move moderately upfield with formation of hydrogen bonds that are stronger than those to solvent water, downfield for those that are weaker, and downfield with the purely electrostatic effects seen with Na^+.[10] The selective downfield change we observe for $Co(NH_3)_6^{3+}$ is therefore consistent with a combination of the strong electrostatic effect of trivalent Co^{3+} and hydrogen bonding to the N7 that is weaker with amine ligands than with solvent water.[21] Thus, among the metals we have studied, the *direction* of chemical shift change is a consequence of each metal's properties, while the degree of selectivity is shown by the *relative magnitude* of the change. The selectivity of binding of $Co(NH_3)_6^{3+}$ to the GG·UU motif is again just the opposite to what we had found for the tandem GA·AG motif, in which we observed a small ($\sim$1 ppm) nonselective downfield change,[11] little more than the change we observed for [7-^{15}N]-guanosine with 10 equiv under the same conditions (Supporting Information). The ligands of $Co(NH_3)_6^{3+}$ are known to exchange extremely slowly,[21] although rare exceptions have been noted,[6] so that RNA is unlikely to coordinate directly to the metal. Our results demonstrate that, although $Co(NH_3)_6^{3+}$ is not attracted to the localized GA·AG site which contains a phosphate (presumably because of the difficulty of inner sphere binding), it is attracted to the GG·UU site.

The G·U base pair is fairly stable, occurs frequently in nature, and is unique in having only hydrogen bond acceptors across the major groove.[22] Thus, tandem G·U pairs create a broad cavity with negative electrostatic potential that is more uniform than that of Watson–Crick pairs, although not necessarily stronger.[23] The original X-ray structure of the P4–P6 domain of the group I intron showed $Os(NH_3)_6^{3+}$ bound to the N7 and O6 atoms of the two guanines of this tandem G·U.[13] Tinoco used ^1H NMR nuclear Overhauser effect cross-peaks to determine the full structure of the hairpin model of the P5b stem loop with $Co(NH_3)_6^{3+}$,[14] and found it to be similar to that in the crystal. $Co(NH_3)_6^{3+}$ and $Mg(H_2O)_6^{2+}$ have been proposed to bind to the same RNA motifs, since they have similar sizes and geometries.[24] This stable hairpin with a defined structure thus presented a good opportunity to compare $Co(NH_3)_6^{3+}$ and $Mg(H_2O)_6^{2+}$ binding in solution using ^{15}N NMR.

The two preformed metal binding motifs we have now investigated by ^{15}N NMR provide strikingly different examples of metal specificity. In the GA·AG motif, the phosphate and a nearby guanine N7 provide ligands that can bind to some transition metals as well as to Mg^{2+}, but not to $Co(NH_3)_6^{3+}$.[11] In the GG·UU motif reported here, the two guanine N7 atoms and the two O6 atoms, but no phosphates, form a broad cavity to which metal hexammines and K^+ are attracted, while $Mg(H_2O)_6^{2+}$ is not, despite the similarity in size between $Co(NH_3)_6^{3+}$ and $Mg(H_2O)_6^{2+}$. Others have noted that $Co(NH_3)_6^{3+}$ is not always a good $Mg(H_2O)_6^{2+}$ mimic.[4,6,25]

Perhaps in vivo, the specific but moderate binding of K^+ in strategically located tandem GU pairs may allow formation of transient associations that are then readily disrupted as the RNA continues to fold. Although the intracellular level of Na^+ is 5–15 mM, that of K^+ is significantly higher, $\sim$140 mM.[26] Because K^+ is a large ion with a particularly loose and flexible hydration layer

that is easily displaced,[19] it fits well into the uniformly negative major groove of the GG·UU motif where it can make direct contacts to the many base electron donors. Our results demonstrate that, although the GG·UU motif is not a binding site for Mg^{2+}, it is a binding site for the other biologically abundant metal ion, K^+.

Supporting Information Available: Experimental methods, partial ^{15}N NMR spectra, tables of ^{15}N NMR chemical shifts for the labeled hairpin and [7-^{15}N]-guanosine, 2D HSQC spectra, ^1H NMR spectra after metal titration, and anion exchange HPLC chromatograms after metal titration. This material is available free of charge via the Internet at http://pubs.acs.org.

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