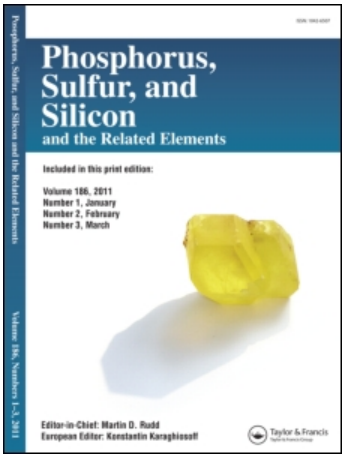

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Divalent Cation Binding to the Well-Conserved Tandem G-A Pairs and Flanking C-G Pair in Hammerhead Ribozyme as Revealed by Heteronuclear NMR Spectroscopy

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DIVALENT CATION BINDING TO THE WELL-CONSERVED TANDEM G-A PAIRS AND FLANKING C-G PAIR IN HAMMERHEAD RIBOZYME AS REVEALED BY HETERONUCLEAR NMR SPECTROSCOPY

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Hammerhead ribozymes have catalytically important tandem G-A pairs (G12-A9 and G8-A13 pairs) and flanking C11.1-G10.1 pair [so called A9-G10.1 motif] in the core region, and the A9-G10.1 motif captures the divalent cation.[1–3] In this study, we measured ^{31}P-, ^{1}H-, and ^{13}C-NMR spectroscopy of the RNA oligomer, GA10: r(GGA**CGA**GUCC)$_2$, to examine whether this motif by itself (in the absence of other catalytic loops) might be sufficient to capture structurally and catalytically important metal ions in solution. GA10 forms a self-associated duplex, and contains tandem GS-A6* pairs and flanking C4-G7* pairs which mimics an A9-G10.1 motif of hammerhead ribozymes. (The residues with "*" belongs to the opposite strand of the duplex.)[4]

Titrations were performed using $MgCl_2$, $CdCl_2$, $NaClO_4$, and $Co(NH_3)_6Cl_3$. Typical acquisition parameters for 1D ^{31}P-NMR spectra were 313 K, a spectral width of 10000 Hz digitized into 16384 points (0.61 Hz/point and 0.0020 ppm/point), and 512 scans were averaged. For the accurate assignment of ^{31}P-resonances, ^{1}H-^{31}P HMQC NOESY spectra[4] were measured at several points during the titration. Other spectra were recorded as described before.[4]

We deduced that the A9-G10.1 motif was able to capture a Mg(II) and Cd(II) ions in solution in the absence of any other part of a hammerhead ribozyme since the chemical shift values of the phosphorus atom of A6

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(P/A6) C8 of G7 (C8/G7), and H8 of G7 (H8/G7), in the A9-G10.1 motif of a model duplex, GA10, were selectively perturbed during titration. Finally, we conclude that the A9-G10.1 motif is sufficient for capture of divalent cations, such as Mg(II) and Cd(II) ions.

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