Quantification and structural characterization of the Mn^{2+} binding sites in the extended hammerhead ribozyme

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Electron Paramagnetic Resonance (EPR) spectroscopy is used to study the binding of Mn^{2+} ions to the extended hammerhead ribozyme (HHRz)[1] and to compare it with the binding to the minimal HHRz.[2,3] Continuous wave EPR measurements show that the extended HHRz possesses a single high-affinity Mn^{2+} binding site with a K_D of 1 nM at an NaCl concentration of 0.1 M. This dissociation constant is three orders of magnitude smaller than the K_D determined for the single high-affinity Mn^{2+} site in the minimal HHRz. In addition, whereas the high-affinity Mn^{2+} is displaced from the minimal HHRz upon binding of the aminoglycoside antibiotic neomycin B it is not from the extended HHRz. Intriguingly, these observations parallel the fact that the extended HHRz is catalytically active at small salt concentrations as encountered in vivo, whereas the minimal HHRz is not.[1] However, despite these pronounced differences in binding, a comparison between the Electron Spin Echo Envelope Modulation and Hyperfine Sublevel Correlation spectra of the minimal and extended HHRz demonstrates that both binding sites are structurally very similar. This suggests that the Mn^{II} is located in both ribozymes between the bases A9 and G10.1 of the sheared G•A tandem basepair, as shown previously and in detail for the minimal HHRz.[3,4] Thus, the much stronger Mn^{2+} binding in the extended HHRz is attributed to the interaction between the two external loops which reduces the RNA dynamics and traps the Mn^{2+} in the tightly bound conformation.

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