

P003 Structure of the high-affinity manganese(II) binding site in a natural hammerhead ribozyme
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Electron Paramagnetic Resonance (EPR) spectroscopy was used to study the binding of Mn^{II} ions to a minimal¹ and a natural hammerhead ribozyme² (HHRz). Continuous wave EPR studies showed that the natural HHRz possesses a single high-affinity Mn^{II} 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 high-affinity Mn^{II} in the minimal HHRz. In addition, whereas the high-affinity Mn^{II} is displaced from its binding site in the minimal HHRz by the aminoglycoside antibiotic neomycin B it is not in the natural HHRz. However, a comparison between the Electron Spin Echo Envelope Modulation and Hyperfine Sublevel Correlation spectra of the minimal and natural HHRz demonstrates that both binding sites are structurally very similar and an analysis of the pulsed EPR spectra in combination with Density Functional Theory calculations on the minimal HHRz showed that the high-affinity Mn^{II} binding site is in both cases located between A9 and G10.1. Thus, the differences in the dissociation constants are attributed to the reduced dynamics of the natural HHRz, due to the loop-loop interaction, which traps the Mn^{II} in the tightly bound conformation.

[1] O. Schiemann, J. Fritscher, N. Kisseleva, S.T. Sigurdsson, T.F. Prisner, *ChemBioChem* 2003, 4, 1057-1065.

[2] A. Khvorova, A. Lescoute, E. Westhof, S.D. Jayasena, *Nature Struct. Biol.* 2003, 10, 708-712.