Structure of the Branchpoint Domain 6 in a Group II Intron Ribozyme

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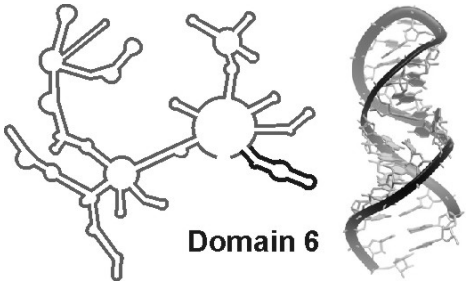
Group II introns are self-splicing catalytic RNAs occurring in plants, fungi, and lower eukaryotes. These molecular machines excise themselves from the primary RNA transcript in a two-step mechanism similar to the spliceosome in higher eukaryotes. Upon formation of the correct three dimensional fold in the presence of K^+ and Mg^{2+}, a 2'-OH of a highly conserved adenosine within domain 6 (D6) acts as the nucleophile in the first step of splicing, forming a 2'-5'-phosphodiester linkage.[1] We have now solved the solution structure of a D6 construct from the intron ai5γ found in the cox 1 gene of yeast.[2] This domain adopts a hairpin structure with two helical regions flanking the branching adenosine. The base pairing we found in solution differs slightly from the previously published X-ray structure,[3] but is consistent with phylogenetic studies and biochemical experiments. In solution, the conserved adenosine moiety is flipped out of the helix and accommodated in the minor groove. This small deviation from helical geometry exposes the 2'-OH to the solvent, thus positioning this functional group for the nucleophilic attack at the 5'-splice site. As divalent metal ions are often directly involved in ribozyme catalysis, we investigated Mg^{2+} binding to D6 by NMR experiments. Strong changes in chemical shifts were observed around the branch site, indicating metal ion binding in this region. Indeed the neighboring GU wobble pairs are particular suitable to bind metal ions, which explains their phylogenetic conservation. Our finding is a further[4] indication for a metal ion binding at the catalytic center of group II introns.



[1] R. K. O. Sigel, *Eur. J. Inorg. Chem.* **2005**, *in press*. P. Z. Qin, A. M. Pyle *Curr. Op. Struct. Biol.* **1998**, *8*, 301. K. Lehmann, U. Schmidt *Crit. Rev. Biochem. Mol. Biol.* **2003**, *38*, 249.
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[3] L. Zhang, J. A. Doudna, *Science* **2002**, *295*, 2084.
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