

Secondary structure confirmation and localization of Mg^{2+} ions in the mammalian CPEB₃ ribozyme.

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Streszczenie

Most of today's knowledge of the CPEB3 ribozyme, one of the few small self-cleaving ribozymes known to occur in humans, is based on comparative studies with the hepatitis delta virus (HDV) ribozyme, which is highly similar in cleavage mechanism and probably also in structure. Here we present detailed NMR studies of the CPEB3 ribozyme in order to verify the formation of the predicted nested double pseudoknot in solution. In particular, the influence of $Mg(2+)$, the ribozyme's crucial cofactor, on the CPEB3 structure is investigated. NMR titrations, Tb(3+)-induced cleavage, as well as stoichiometry determination by hydroxyquinoline sulfonic acid fluorescence and equilibrium dialysis, are used to evaluate the number, location, and binding mode of $Mg(2+)$ ions. Up to eight $Mg(2+)$ ions interact site-specifically with the ribozyme, four of which are bound with high affinity. The global fold of the CPEB3 ribozyme, encompassing 80%-90% of the predicted base pairs, is formed in the presence of monovalent ions alone. Low millimolar concentrations of $Mg(2+)$ promote a more compact fold and lead to the formation of additional structures in the core of the ribozyme, which contains the inner small pseudoknot and the active site. Several $Mg(2+)$ binding sites, which are important for the functional fold, appear to be located in corresponding locations in the HDV and CPEB3 ribozyme, demonstrating the particular relevance of $Mg(2+)$ for the nested double pseudoknot structure.

Słowa kluczowe

CPEB3, NMR spectroscopy, metal ion, pseudoknot, ribozyme

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