Unusual metal specificity and structure of the group I ribozyme of Chlamydomonas reinhardtii 23S rRNA

郭泰志

Kuo TC;Odom OW;Herrin DL;;

摘要

Group I intron ribozymes require cations for folding and catalysis, and the current literature indicates that a number of cations can promote folding, but only Mg2+ and Mn2+ support both processes. However, some group I introns are active only with Mg2+, e.g. three of the five group I introns in Chlamydomonas reinhardtii. We have investigated one of these ribozymes, an intron from the 23S LSU rRNA gene of Chlamydomonas reinhardtii (Cr.LSU), by determining if the inhibition by Mn2+ involves catalysis, folding, or both. Kinetic analysis of guanosine-dependent cleavage by a Cr.LSU ribozyme, 23S.5ΔGb, that lacks the 3' exon and intron-terminal G shows that Mn2+ does not affect guanosine binding or catalysis, but instead promotes misfolding of the ribozyme. Surprisingly, ribozyme misfolding induced by Mn2+ is highly cooperative, with a Hill coefficient larger than that of native folding induced by Mg2+. At lower Mn2+ concentrations, metal inhibition is largely alleviated by the guanosine cosubstrate (GMP). The concentration dependence of guanosine cosubstrate-induced folding suggests that it functions by interacting with the G binding site, perhaps by displacing an inhibitory Mn2+. Because of these and other properties of Cr.LSU, the tertiary structure of the intron from 23S.5ΔGb was examined using Fe2+-EDTA cleavage. The ground-state structure shows evidence of an unusually open ribozyme core: the catalytic P3-P7 domain and the nucleotides that connect it to the P4-P5-P6 domain are exposed to solvent. The implications of this structure for the in vitro and in vivo properties of this intron ribozyme are discussed