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Crystal Structures of ColE7 in Complex DNA/Zn^{2+} and Im7/Ni^{2+} Show How a Transition Metal Ion Bound ColE7 Binds and Cleaves DNA. L.G. Doudeva[1], H. Huang, Z. Shi[1], C.-L. Li[1], W.-C. Chu[2], H.S. Yuan[1], [1]Inst. of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC, [2]Institutes of Biomedical Engineering, National Yang Ming Univ., Taiwan, ROC.

The nuclease domain of ColE7 (N-ColE7) contains an H-N-H motif which folds in a metal finger topology. Here we report the crystal structures of a Zn^{2+}-bound N-ColE7 (H545E mutant) in complex with a 12-bp duplex DNA (5'-CGGGATATCCCG-3') and a Ni^{2+}-bound N-ColE7 in complex with the inhibitor Im7 at a resolution of 2.5 Å and 2.0 Å, respectively. Metal-dependent cleavage assays and site-directed mutagenesis showed that N-ColE7 cleaves double-stranded DNA with a single metal ion cofactor, Ni^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}. In the crystal structure of N-ColE7-DNA complex, the zinc ion is directly coordinated to three histidines and the DNA scissile phosphate in a tetrahedral geometry. In contrast, Ni^{2+} is bound in N-ColE7 in two different modes, to four ligands (three histidines and one phosphate ion), or to five ligands with an additional water molecule. These data suggest that the divalent metal ion in the His-metal finger motif can be coordinated to six ligands, such as Mg^{2+} in I-PpoI, *Serratia* nuclease and Vvn, five ligands or four ligands, such as Ni^{2+} or Zn^{2+} in ColE7. Universally, the metal ion in the His-metal finger motif is bound to the DNA scissile phosphate and serves three roles during hydrolysis: polarization of the P-O bond for nucleophilic attack, stabilization of the phosphoanion transition state and stabilization of the cleaved product.