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X-Ray Spectroscopy of Zn Enzymes

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ABSTRACT: The ligation state of the single zinc site in primase from *Escherichia coli* changes when various substrates and cofactors are added alone or in combination as determined by X-ray absorption spectroscopy. The zinc site in native primase and in primase bound to low (30 mM) magnesium acetate was found to be tetrahedrally ligated by three sulfurs at an average distance of 2.36 ± 0.02 Å and one histidine nitrogen located at a distance of 2.15 ± 0.03 Å. When ATP, ATP and (dT)₁₇, or ATP, low magnesium acetate and (dT)₁₇ was added to primase, one (two) additional nitrogen/oxygen ligands were coordinated to the zinc together with the histidine nitrogen at an average distance of 2.15 ± 0.03 Å. These additional ligands are likely from adjacent phosphates from ATP. Another structure was observed for the primase-(dT)₁₇ complex in which an additional nitrogen/oxygen ligand likely from the phosphate backbone together with the histidine nitrogen were located at a significantly shorter average distance of 2.05 ± 0.03 Å. High magnesium acetate (300 mM) completely inactivates primase in a reversible manner such that the region near the zinc ligands become accessible to proteolytic digestion [Urlacher, T. M., and Griep, M. A. *Biochemistry* 34: 16708-16714, 1995]. In this inactive complex, additional oxygen/nitrogen ligands from acetate as well as the histidine nitrogen are located at a distance of 2.20 ± 0.03 Å from the zinc site. To test whether the catalytic magnesium was binding within ~ 5 Å of the zinc, primase was incubated with high (300 mM) manganese acetate. The functional properties of magnesium and manganese are similar but the larger atomic number of manganese enhances the x-ray backscattering making it possible to identify. Since no significant difference was observed from the manganese incubated sample, the catalytic metal binding site is likely located > 5 Å from the zinc. These studies clearly show that primase zinc ligation changes upon binding substrates.