Characterization of the Transmembrane Metal binding site in ZntA

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ZntA from *Escherichia coli*, a Zn(II), Pb(II) and Cd(II)-transporting ATPase, belongs to the family of P_{1B}-type ATPases that transport heavy metals. It contains a hydrophilic N-terminal domain that binds a single metal ion and a transport domain made up of eight transmembrane helices and a cytosolic ATP-binding loop. In order to investigate the metal binding properties of the transport domain, we characterized a mutant of ZntA, ΔN-ZntA, lacking the first 106 amino acids and the N-terminal metal binding site. ICP-MS results showed that ΔN-ZntA can bind a variety of metals with a stoichiometry of 1. The affinity of Zn(II), Pb(II) and Cd(II) for this transmembrane metal site in ΔN-ZntA was determined by competitive titration with the metallochromic indicator, meg-fura-2. The association constants were ~ $2 - 12 \times 10^8$ M^{-1}. Site-specific mutagenesis confirmed that two cysteine residues, part of the distinct CPC motif conserved in TM6 of many P_{1B}-type ATPases, supply two of the ligands to the metal. In addition, site-specific mutagenesis shows that an aspartate residue in TM8, which is conserved in Zn(II)/Pb(II)/Cd(II)-transporting ATPases, but not in Cu(I)-transporting ATPases, provides ligands to the transmembrane metal site. Thus, this aspartate residue may help to determine specificity of metal binding to the transmembrane site.