Metal Binding Studies of the Amino Terminal Domain of ZntA, a Zn/Pb/Cd-Transporting ATPase

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ZntA, a P_{1B}-type ATPase from *Escherichia coli*, mediates resistance specifically to Pb, Cd and Zn by transporting these metals out of the cytoplasm. ZntA has two metal binding sites, one in the hydrophilic N-terminal domain and the second in the transmembrane domain. The N-terminal domain has ~120 residues with one copy of the GXXCXXC motif, which is a signature metal-binding motif found in many P_{1B}-type ATPases, and is sufficient for binding Zn and Cd. In addition, it also has a CCX(D/E)XXC motif, which is required for binding Pb. The function of this domain (N1-ZntA) in the overall mechanism is not yet known. We propose that it binds metal ions efficiently from solution and transfers it to the transmembrane domain.

Binding of Pb(II) and Cd(II) to N1-ZntA produce metal-thiolate charge-transfer peaks in the 250-400 nm range. These charge-transfer spectra were used to measure binding affinity by direct titration and binding kinetics by stopped-flow techniques. The association constant for binding was ~ 10^7 M^{-1}, while the rate constant of metal binding, k_{on} was surprisingly high, ~ 10^7 $M^{-1}s^{-1}$ at 4. This large rate constant for metal binding to the N-terminal domain of ZntA compared to its low turn over rate, indicates that this step is not rate limiting in the overall transport mechanism. The amino-terminal domain has a single tryptophan residue; upon metal binding, its fluorescence emission decreases by ~25-30%. This change in the fluorescence signal was also used to measure the association constant for a variety of metals, both substrates for the pump and otherwise.

The metal site in the N-terminal domain is being currently characterized in the full-length transporter in which the transmembrane site has been knocked out by mutagenesis. Structural studies of N1-ZntA are also underway to provide insight into different metal binding geometry displayed by Pb and Zn/Cd at the N-terminal site.