Crystal Structure of the *S. aureus* pI258 CadC Cd(II)/Zn(II)/Pb(II) Responsive Repressor

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The *Staphylococcus aureus* plasmid pI258 *cadCA* operon encodes a P-type ATPase, CadA, that confers resistance to the heavy metals Cd(II) and Pb(II). Expression of this heavy metal efflux pump is regulated by CadC, a homodimeric repressor that dissociates from the *cad* operator/promoter upon binding of Cd(II), Pb(II) or Zn(II). CadC is a member of the ArsR/SmtB family of metalloregulatory proteins. The crystal structure of CadC has been solved to 1.9 Å. In the dimer there are four metal bindings, two each of two types of sites. The type 1 site is the physiological inducer binding site. Each type 1 metal binding site is formed from the N-terminus of one monomer and Cys-58 and Cys-60 of the other. In the crystal structure, the type 1 site is are sterically hindered by Tyr-12, which forms a hydrogen bond with Cys-58, preventing binding of metals to Site 1. The type 2 metal binding sites are formed at the dimerization interface by Asp-101 and His-103 in one monomer and His-114 and Glu-117 in the other. Interestingly, the two type 2 sites have bound Zn ions, even though, no additional Zn(II) was added to the crystallization setups. In the present study, the role of each site was investigated by mutagenesis. Site I mutations were constructed to measure the metal binding capacity in both Site1 and 2. Tyr-12 was mutated to Phe and Ala to eliminate hydrogen bonding to Cys-58. The crystal structure of Y12F mutant revealed that steric hindrance by the bulky phenylalanine residue, and not hydrogen bonding, blocks metal binding. The structure of the Y12A mutant is in progress, and we predict that there will be a large conformational change upon metal binding in this mutant. Site 2 mutations (His-103 to Ala and Asp-101 to Gly) were constructed to investigate the function of Zn(II) in dimerization. In the crystal structure of these mutant proteins, no Zn(II) was observed at the dimer interface. The data suggest that Zn(II) in the dimerization domain is not essential for dimer formation. Direct measurement of metal binding by ICP-MS showed that both Site 1 and Site 2 can bind either Cd(II) or Zn(II). However, Site 1 has higher affinity for Cd(II) over Zn(III), and Site 2 prefers Zn(II) over Cd(II). *This work is supported by NIH grant AI45428.*