Coordination Chemistry of Metal Sensing Sites and Allosteric Switching in Metal-responsive Transcriptional Regulators

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The ArsR/SmtB family of homodimeric "winged" helix transcriptional repressors allows pathogenic bacteria to specifically respond to (or *sense*) stress induced by toxic concentrations of essential metal ions, as well as heavy metal pollutants. These proteins repress the expression of operons encoding specific metal chelators and efflux pumps; upon direct binding of specific metals, the expression of the operon is derepressed. Comparative structural, spectroscopic and mutagenesis studies of various ArsR/SmtB family members including *S. aureus* CzrA (a Zn/Co sensor), *M. tuberculosis* NmtR (a Ni/Co sensor) and *S. aureus* pI258 CadC (a Cd/Pb/Bi sensor) have led us to hypothesize that coordination geometry is a primary determinant for functional metal selectivity and allosteric regulation of DNA binding by metal sensor proteins. Recent findings from ^{111m}Cd perturbed angular correlation (PAC) and ^{113}Cd NMR spectroscopies of two new ArsR/SmtB sensors *Anabaena* AztR, a newly discovered Zn/Pb/Cd sensor, and *M. tuberculosis* CmtR, a Cd/Pb sensor, will be discussed. *Anabaena* AztR regulates *aztA* expression, encoding a Zn/Pb-specific efflux pump. AztR forms a distorted S_3N α3N metal coordination complex (^{113}Cd δ=609 ppm; ω_o=0.185 rad/ns, η=0.23 from PAC spectroscopy). In the case of CmtR, the Cd(II) ion is bound by just two strongly bound thiolate ligands, Cys57/Cys61 (K_{Cd}=1.7x10^{12} M^{-1}; ^{113}Cd δ=480 ppm; PAC parameters ω_o=0.291 rad/ns, η=0.18). A third conserved cysteine, Cys102, appears only weakly coordinated, but plays a critical role in allosteric regulation of *cmt* operator-promotor (O/P) binding, since substitution of Cys102 abrogates disassembly of oligomeric CmtR-*cmt* O/P complexes. Zn(II) is also a strong regulator of DNA binding of CmtR *in vitro*, yet does not induce in the operon *in vivo*. Both AztR and CmtR Cd(II) chelates are structurally distinct from the strongly distorted tetrathiolate α3N site of CadCs (^{113}Cd δ=622 ppm; ω_o=0.110 rad/ns, η=0.45). Despite distinct coordination chemistries, a common theme that emerges is that these metal-sensing chelates have at least one open coordination site or rapidly exchanging metal ligand that might facilitate metal-ligand exchange reactions important for metal resistance *in vivo*. The evolutionary implications of these structural findings will be discussed. Supported by NIH Grant GM042569.