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 \textit{S. aureus} CzrA (a Zn/Co sensor), \textit{M. tuberculosis} NmtR (a Ni/Co sensor) and \textit{S. aureus} pl258 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 \textsuperscript{111m}Cd perturbed angular correlation (PAC) and \textsuperscript{113}Cd NMR spectroscopies of two new ArsR/SmtB sensors \textit{Anabaena} AztR, a newly discovered Zn/Pb/Cd sensor, and \textit{M. tuberculosis} CmtR, a Cd/Pb sensor, will be discussed. \textit{Anabaena} AztR regulates \textit{aztA} expression, encoding a Zn/Pb-specific efflux pump. AztR forms a distorted S\textsubscript{2}N\textalpha3N metal coordination complex (\textsuperscript{113}Cd \(\delta=609\) ppm; \(\omega_o=0.185\) rad/ns, \(\eta=0.23\) from PAC spectroscopy). In the case of CmtR, the Cd(II) ion is bound by just two strongly bound thiolate ligands, Cys57/Cys61 (\(K_{\text{Cd}}=1.7\times10^{12}\) M\textsuperscript{-1}; \textsuperscript{113}Cd \(\delta=480\) ppm; PAC parameters \(\omega_o=0.291\) rad/ns, \(\eta=0.18\)). A third conserved cysteine, Cys102, appears only weakly coordinated, but plays a critical role in allosteric regulation of \textit{cmt} operator-promotor (O/P) binding, since substitution of Cys102 abrogates disassembly of oligomeric CmtR-\textit{cmt} O/P complexes. Zn(II) is also a strong regulator of DNA binding of CmtR \textit{in vitro}, yet does not induce in the operon \textit{in vivo}. Both AztR and CmtR Cd(II) chelates are structurally distinct from the strongly distorted tetrahedral \textalpha3N site of CadCs (\textsuperscript{113}Cd \(\delta=622\) ppm; \(\omega_o=0.110\) rad/ns, \(\eta=0.45\)). 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 \textit{in vivo}. The evolutionary implications of these structural findings will be discussed. Supported by NIH Grant GM042569.