Structural Studies of Ni Trafficking Proteins: NikA, NikR and HypA

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NikA is a periplasmic binding protein associated with the Ni-specific transporter, NikABCDE in *E. coli.* Early investigations of the Ni site structure by XAS showed $(N/O)_{6\text{-}7}S$ coordination.[1] Recent refinements are consistent with the involvement of 2 - 3 His residues. The data are consistent with a $Ni(N/O)_4(His)_2$ site. This contrasts with a recent crystal structure, that shows Ni bound with five aqua ligands at an average Ni-O distance of 2.7 Å.[2]

HypA is an accessory nickel protein that assists in the incorporation of nickel in hydrogenase in *E. coli* and both urease and hydrogenase in *Helicobacter pylori*.[3] HypA contains two metal centers, one nickel and one zinc. Both sites have been investigated in *H. pylori* HypA by XAS. XANES analysis shows that the Ni site is 6-coordinate with a $(N/O)_6$ ligand donor atom set, of which *ca.* two ligands are provided by His residues. In the absence of Ni, the Zn site is 4-coordinate with 3 S-donors and one N/O-donor. Interestingly, upon coordination of Ni, the Zn site changes to a site containing S_4 coordination. This change in coordination likely plays an important role in the function of HypA within the cell and will be discussed.

NikR functions as a transcriptional regulator. The tetrameric protein contains four high-affinity metal binding sites that are 4-coordinate with a $(N/O)_3S$-ligand donor set in its free state, switching to 6-coordinate, with $(N/O)_6$-donors, upon binding to operator DNA.[4] Binding additional M^{2+} ions in two lower-affinity sites/tetramer in the presence of DNA reverts the coordination chemistry of the Ni to its $(N/O)_3S$ ligation state. Thus, changes in salt concentration may effect the coordination chemistry of the high-affinity nickel site, and can be examined using XAS. The structural response of the high-affinity site to DNA and M^{2+} ions likely plays a role in the strength (or degree) of the transcriptional response exhibited by NikR in vivo.

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