

A High-affinity Ni(II)-Binding Site from *Escherichia coli* HypB

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The biosynthesis of metalloenzymes often requires the use of metallochaperones to carefully escort a specific metal to its functional site. A prime example is the maturation of [NiFe]-hydrogenase, an enzyme expressed in many bacteria such as *E. coli* and *H. pylori*, that is responsible for the reversible formation of two protons and two electrons from hydrogen gas (1). The electrons produced are often shuttled off into other parts of the cell driving additional cellular functions. The delivery of Ni(II) into the active site of the [NiFe]-hydrogenase is a process that requires the accessory protein HypB. This protein contains two metal-binding sites, one of which demonstrates a high-affinity for nickel using an N-terminal CXXCGC motif (2).

By using the facilities at both the Stanford Synchrotron Radiation Laboratory (SSRL) and the Canadian Light Source (CLS) we performed X-ray absorption spectroscopy (XAS) on Ni(II)-loaded HypB. This technique allowed us to identify the amino acid ligands that bind the Ni(II) ion in addition to the coordination geometry surrounding the metal. Additional XAS experiments were carried out on a novel fusion protein produced by fusing the first ten amino acids of HypB to ubiquitin, a scaffold lacking any metal binding properties. A small peptide consisting of the first nine amino acids of HypB was also prepared and analyzed. Both of these constructs were able to bind Ni(II) with a similar affinity and displayed identical spectroscopic properties indicating an identical structural environment for Ni(II). These experiments have not only provided valuable information on HypB but allowed us to define a minimal metal-binding site for Ni(II) with possible antibiotic capabilities.

1. S. Lutz et. al. (1991) *Mol. Microbiol.* 5,123-35.
2. M. R. Leach et. al. (2005) *Biochemistry*, 44, 12229-38.