Nickel Coordination Chemistry and Regulation of DNA Binding in *E. coli* and *H. pylori* NikR

Sharon LaMont Leitch,[1] Jeffrey S. Iwig,[2] Erin L. Benanti,[2] David Kennedy,[1] Peter T. Chivers,[2] and Michael J. Maroney[1]

[1]Department of Chemistry, University of Massachusetts, [2]Department of Biochemistry and Molecular Biophysics, Washington University

NikR is a transcriptional regulator found in bacteria and archaea that is activated in response to high intracellular nickel concentrations. The tetrameric protein contains four high-affinity metal binding sites and two lower affinity sites. The high-affinity site of *E. coli* and *H. pylori* NikR have been studied using x-ray absorption spectroscopy and other techniques.[1] XAS shows that the high-affinity site contains a square planar Ni site with 3N/O-donors (including at least 2 His) and one cysteine S-donor. This site becomes six-coordinate with exclusively N/O-ligation upon binding the protein to operator DNA.[1] Recent studies provide insight on the role of metal binding and DNA binding interactions. XAS has been used to demonstrate that the geometry and ligand selection of Co(II), Cu(II), Cu(I) and Zn(II) varies, and is consistent with a model where the protein responds to the ligand selection and geometry in a way that produces a unique protein conformation for the nickel complex. Binding Co(II) to the low affinity sites provides a probe of the structure of this site and its effect on the high-affinity site. The data show a tetrahedral site composed of N/O-donors. With Co bound, the Ni high affinity site is four-coordinate and planar even in the presence of operator DNA.

XAS studies of *H. pylori* NikR show that the high-affinity metal binding site is very similar to that found in *E. coli* NikR and also exhibits a change in nickel coordination upon binding to DNA. The high-affinity nickel binding site shows a four coordinate planar geometry with 3 N/O- and 1 S-donor. Upon addition of operator fragments, the coordination rearranges to a site best described as five- or six-coordinate with a ligand environment composed of N/O-donors, at least 2 of which are imidazole ligands. A deletion mutant of *H. pylori* with the first 9 amino acids (nt9) removed has also been studied with XAS. The effects on the high-affinity site upon mutation of these residues, which affect differential DNA binding, will be discussed.

1. Carrington, P. E.; Chivers, P. T.; Al-Mjeni, F.; Sauer, R. T.; Maroney, M. J., "Nickel coordination is regulated by the DNA-bound state of NikR." *Nat Struct Biol* **2003,** 10, (2), 126.