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# Investigation of the Role of Nickel in the Regulation of the Cell Cycle

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*Acireductone dioxygenases (ARDs) are enzymes involved in cell cycle regulation. The ARDs produced by a bacterium called Klebsiella pneumoniae have been investigated in detail, but the precise function of one of them, a nickel-containing ARD (Ni-ARD), is unclear. Scientists from the University of Massachusetts in Amherst and Brandeis University in Waltham, Massachusetts, have examined the structure of the nickel-containing active site of Ni-ARD, in the presence and absence of substrate. The results of the study provide the first structural details of the nickel site in the only known nickel-containing dioxygenase, and provide insight into substrate binding and the role of nickel in catalyzing substrate oxidation.*

Polyamines are molecules that are critical for proper regulation of the cell cycle. Addition of polyamines to cells accelerates their DNA replication and division, whereas inhibition of polyamine biosynthesis arrests DNA replication and prevents continuation of the cell cycle.

Methylthioadenosine (MTA) is a strong inhibitor of polyamine biosynthesis and transmethylation reactions, so the concentration of MTA in biological systems is tightly regulated. This control is achieved through a regulation mechanism called the methionine salvage pathway, where MTA is recycled through a series of reactions that convert it to methionine.

In *Klebsiella pneumoniae*, a bacterium causing pneumonia and urinary tract infection, the pathway has been investigated in detail, and all its intermediates have been identified. One of the intermediates of the pathway, called acireductone, is catalyzed by two enzymes called acireductone dioxygenases (ARDs). The two enzymes share a common protein and differ only in the metal ion present, which is either iron or nickel.

Reaction of acireductone with iron-ARD and oxygen produces a precursor of methionine, while reaction of acireductone with nickel-ARD does not recycle MTA to methionine. The precise function of the nickel-ARD reaction in *K. pneumonia* is unclear, and may be considered a shunt in the methionine salvage pathway, aiding in the regulation of methionine. So, we decided to investigate the structure of the catalytic nickel center in resting nickel-ARD enzyme and the enzyme-substrate complex, by using x-ray absorption spectroscopy at beamline X9B of the National Synchrotron Light Source at Brookhaven National Laboratory. We considered the following substrates: acireductone, carbon(14)-labeled substrates or substrate analogues, and oxygen(18).

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X-ray absorption spectroscopy data obtained on the enzyme-substrate complex show that the substrate binds to the nickel binding site via two contact sites by displacing two ligands, at least one of which is a histidine ligand. These results show that the nickel-ARD binds to a substrate by activating its oxidation by oxygen.

Analyses of x-ray absorption near-edge spectroscopy (XANES) and extended x-ray absorption fine structure spectroscopy (EXAFS) data show that the structure of the nickel binding site in the resting enzyme consists of six nickel centers with six oxygen or nitrogen ligands in each center. A schematic representation of a nickel center is provided in **Figure 1.**

A comparison of the EXAFS spectra for resting nickel-ARD and the nickel-ARD en-

zyme-substrate complex (**Figure 2**, top-right and bottom-right spectra) reveal changes implying that the substrate binds to the nickel site. One of these changes is a dramatic decrease in the intensity of features at a distance of 3 to 4 angstroms, which we believe are due to the presence of histidine imidazole ligands, suggesting that one or more histidine ligands are displaced when a substrate binds to nickel-ARD.

Dioxygenases are known to operate by two distinct types of mechanism: One involves a redox active metal center to activate oxygen to oxidize the substrate. The second mechanism involves activation of the substrate via coordination to the metal center in order to activate it toward oxidation by dioxygen. This latter mechanism, where the metal center need not be redox active, is supported by the XAS results.

We are currently investigating the use of nuclear magnetic resonance methods to characterize in more detail metal-bound substrate species in iron- and nickel-ARDs, and to understand better the differences between the differing activities of these two enzymes.

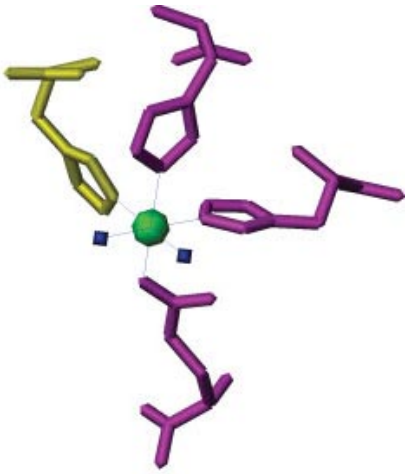

***Figure 1.*** *Nickel site structure in nickel-ARD, drawn from information obtained from x-ray absorption spectroscopy and nuclear magnetic resonance. The nickel ion (green) is surrounded by three histidine residues (purple), a carboxylate residue (yellow) and two non-protein ligands (blue squares), presumably water and/or hydroxide.*

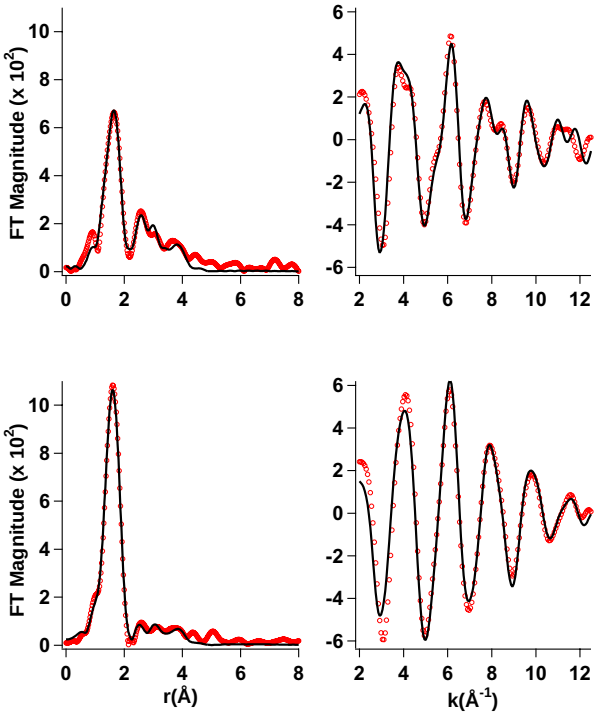


***Figure 2.*** *Nickel K-edge EXAFS spectra. Fourier-tranformed (left) and Fourier-filtered (right) spectra of the resting nickel-ARD (top) and the nickel ARD-substrate (bottom) of* Klebsiella pneumoniae*. Data points are represented by red open circles and the fit by a solid line.*