Nickel has been shown to be an essential trace element involved in the metabolism of several species of bacteria, archaea, and plants. In these organisms, nickel is involved in enzymes that catalyze both non-redox (e.g., urease, glyoxalase I) and redox (e.g., dehydrogenase, carbon monoxide dioxygenase) reactions, and proteins involved in the transport, storage, metallocenter assembly, and regulation of nickel concentration have evolved. Studies of structure/function relationships in nickel biochemistry reveal that cysteine ligands are used to stabilize the Ni(II/III) redox couple. Certain nickel compounds have also been shown to be potent human carcinogens. A likely target for carcinogenic nickel is nuclear histone proteins. Here we present X-ray absorption spectroscopic studies of a model Ni peptide designed to help characterize the structure of the nickel complexes formed with histones and place them in the context of nickel structure/function relationships, to gain insights into the molecular mechanism of nickel carcinogenesis. Key words: carcinogenesis, enzyme, histone, nickel, nucleosome, protein, XAS. Environ Health Perspect 110(suppl 5):705–708 (2002).

Use of XAS for the Elucidation of Metal Structure and Function: Applications to Nickel Biochemistry, Molecular Toxicology, and Carcinogenesis

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Nickel is an essential metal for many archaean, bacterial, and plant species (1), and may yet be found to play a role in the metabolism of higher organisms. Most bacteria and archaean that can live under anaerobic conditions (including beneficial bacteria in the human gut) produce several enzymes that require Ni. Yet, under some circumstances, Ni is a potent human carcinogen (2,3). As potential specific molecular targets for carcinogenic Ni are identified, it becomes possible to examine the structure of these Ni sites for clues to how they give rise to cancer. X-ray absorption spectroscopy (XAS) is one technique that provides detailed structural information regarding biological Ni sites. Analysis of Ni K-edge X-ray absorption near-edge structure (XANES) produces information regarding the coordination number and geometry of a Ni site (4). Comparison of Ni K-edge energies may reveal redox-active Ni sites. The analysis of extended X-ray absorption fine structure (EXAFS) provides information regarding the types of ligands bound to Ni and metric details for the Ni site (5,6). The main goal of this research is to provide structural information regarding biological Ni sites that are potentially involved in carcinogenesis, and to elucidate the likely properties that give rise to carcinogenesis by comparing these structures with those of Ni-containing biological molecules with known functions.

Structure/Function Relationships in Nickel Metallobiochemistry

A variety of Ni-binding proteins and Ni-dependent enzymes have been structurally characterized (Figure 1). As with most transition metals, the uptake, transport, storage, intracellular concentration, and biosynthesis of Ni metalloenzymes are tightly regulated by proteins that specifically bind Ni(II). These proteins include examples of Ni-specific permeases (e.g., Ni2+, HoxN, NikA–NikE) (7), metallochaperones (e.g., UreE, HypB, Cool) (8–10), and proteins involved in the regulation of biosynthesis (e.g., NikR, H3 sensor) (11–13). One member of this group of proteins has been crystals pharmacologically characterized. The metallochaperone UreE was recently shown to contain two Ni-binding domains: a C-terminal polyhistidine domain involved in storing Ni, and an N-terminal domain that serves as a Ni chaperone. This domain binds to the active site of urease (14). A recent crystal structure of the N-terminal domain of Cu2+ ions bound indicates that pairs of His residues are involved in binding Ni in these sites (15). XAS studies of truncated and holo proteins also reveal the importance of His ligands in constructing both the storage and chaperone sites (16–18).

An interesting example of Ni-specific uptake and the regulation thereof is provided by the Nik system in Escherichia coli (7,19). The Nik system comprises five proteins (NikA–NikE) that constitute a Ni-specific ATP-dependent permease, and NikR, a transcription factor that regulates the biosynthesis of the Nik permease in response to intracellular Ni concentration (11,20,21). Of the five proteins comprising the Nik permease, NikA is known to play an important role both in binding Ni and in Ni negative chemotaxis (19,22–25). Deletion of the gene encoding this protein eliminates Ni uptake and therefore the production of active hydrogenases found in E. coli. The Ni-binding site in NikA has been investigated by XAS, which showed that the Ni bound to NikA is in a six-coordinate environment featuring a combination of O/N-donor ligands (26). Given the high carboxylate acid content of NikA and the similarity of the XAS spectra to those obtained from a model compound with carboxylate ligation, Ni(sal)(OH2)2, the data pointed to a structure with several carboxylate ligands. A recent fit of the EXAFS data using multiple scattering theory shows that the data are consistent with a site composed of two or three His imidazole ligands and three or four carboxylates (27).

The biosynthesis of the Nik permease is regulated at the transcriptional level by Fnr (fumarate nitrate regulatory protein), which activates transcription in the absence of oxygen, and by NikR, which represses the transcription of the permease in response to high (micromolar) concentrations of Ni in vitro (11,21). Optimal transcription is thereby achieved when the cells have a high demand for Ni—under anaerobic conditions with low concentrations of Ni.

NikR is a member of the ribbon–helix–helix family of transcription factors (20). These proteins feature homologous N-terminal domains that bind to DNA, and can have specialized C-terminal domains. The details of the DNA-binding mechanism are not completely understood. Of the known ribbon–helix–helix transcription factors, NikR is the only example that is known to respond to metal concentration. NikR is a homodimeric protein that binds as a dimer of dimers to a dyad-symmetric DNA-binding site containing a GTATGA recognition sequence repeated with separation of 16 bases. Proteins with high sequence homology to E. coli NikR have been identified in nine bacterial and six archael species. These proteins reveal the presence of six highly conserved residues (H–X13–X–X–X–X–C–X–E) in the C-terminal domain of NikR that are associated with the presence of a high-affinity

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Featuring two histidine ligands, one S-donor DNA has a planar four-coordinate Ni site indicates that the protein in the absence of XAS analysis examining the high-affinity Ni binding site in NikR. Preliminary binding sites would be sensed by the high-affinity Ni site is implicated. Alternatively, the distribution of Ni in the cell may be under kinetic control, in which case saturation of target Ni-binding sites would be sensed by the high-affinity Ni-binding site in NikR. Preliminary XAS analysis examining the high-affinity Ni site indicates that the protein in the absence of DNA has a planar four-coordinate Ni site featuring two histidine ligands, one S-donor ligand (presumably cysteine), and one other O- or N-donor ligand. Thus, not all of the conserved His residues are involved in forming the high-affinity site.

A predominance of His and carboxylate ligation (Asp and Glu residues) is also found in Ni enzymes that catalyze nonredox processes. E. coli glyoxalase I catalyzes the isomerization of the hemimercaptal formed from the nonenzymatic reaction of glutathione and methylglyoxal into the thioester of D-lactate, which is subsequently hydrolyzed. This provides a mechanism for the detoxification of methylglyoxal by converting it to D-lactate. Crystallographic and XAS data show that the Ni site comprises two His, two Glu, and two aqua/hydroxo ligands (6,28,29).

Urease provides another well-studied example of the use of Ni in a nonredox enzyme (30). Urease is a hydrolytic enzyme that catalyzes the conversion of urea to ammonium ion and carbamate. Here, a dinuclear Ni site is bound to the protein by a combination of four His ligands and a bidentate Asp residue and bridged by a carbamate ligand formed by posttranslational carboxylation of a Lys residue (31). Each Ni is five or six coordinate, with the remaining ligands derived from water molecules in the active site (32–34).

In contrast to enzymes that catalyze nonredox reactions, cysteine ligands play a dominant role in constructing redox-active Ni sites, particularly those that use the formal Ni(III/II) redox couple (Figure 1). Hydrogenases catalyze the two-electron redox chemistry of H₂. The crystal structures of various hydrogenases reveal that the Ni-containing hydrogenases have an active-site Ni center ligated by four cysteines, two of which bridge to the Fe atom in hetero dinuclear active sites (35,36). In the oxidized forms of these enzymes, there is a third OH bridging group that is lost upon reductive activation of the enzyme (37). The recent crystal structures of Carboxydothermus hydrogenoformans (38) and Rhodospirillum rubrum (39) carbon monoxide dehydrogenases, enzymes that catalyze the two-electron redox interconversion of CO and CO₂, reveal a NiFe₅S₉ active-site cluster that incorporates a NiS₄ center formed by one cysteinato and three sulfido ligands. Superoxide dismutases catalyze the conversion of superoxide ion to oxygen and hydrogen peroxide using one-electron redox chemistry. XAS studies of Ni superoxide dismutase (NiSOD) from Streptomyces scouleri shows that the Ni center giving rise to the oxidation and reduction of O₂⁻ is ligated by three S-donor ligands (40). This is true both for the resting, oxidized state, which contains a five-coordinate Ni center with one N-donor and one N- or O-donor ligand in addition to the S-donors, and for the reduced state, which has a four-coordinate Ni site with only one non-S-donor ligand. The amino acid sequence of this enzyme reveals that the protein contains only three S atoms corresponding to residues Cys₂₅, Cys₆₉, and Met₂₈. Although the involvement of the Met residue in the NiSOD active site has not been established, negatively charged S-donor ligands (thiolate and sulfide) clearly stabilize the potential of the Ni(III/II) redox couple from over +1 V for aqueous Ni ions to physiologically accessible redox potentials.

**Nickel Carcinogenesis: A Structure/Function View**

The details of molecular mechanism(s) that give rise to Ni carcinogenesis are not well known. Studies of Ni toxicology and carcinogenesis reveal that Ni is predominantly genotoxic and not mutagenic. These studies
also show that carcinogenic Ni compounds selectively damage heterochromatin, leading to chromatin condensation and enhanced DNA methylation that silences reporter genes when they are located near heterochromatin in yeast or mammalian cells (41). There are several ways in which Ni might gain entrance into the cell. Soluble Ni compounds, which do not appear to be the principle source of carcinogenic Ni, could be transported into the cell via the Mg\textsuperscript{2+} transport system because of the similar charge/size ratio of the two metal ions. However, the concentration of Ni that would be required to effectively compete with Mg\textsuperscript{2+} transport is very high (\textmu M) (2). Insoluble Ni compounds have been demonstrated to enter cells via endocytosis (3). The ability of Ni compounds to transform cells has been correlated to properties that increase endocytosis (e.g., particle size, charge, and solubility). Such a process is also consistent with the predominance of nasal and lung cancers caused by inhalation of insoluble Ni compounds, particularly Ni\textsubscript{3}S\textsubscript{2}, a potent carcinogen (2).

The endocytic vesicles formed may be acidified by fusion with lysosomes, ultimately releasing large quantities of Ni ions into the cytoplasm. Further, fusion of vesicles to the nuclear membrane may deliver large quantities of Ni ions to the nucleus. Once in the nucleus, potential gene silencing, DNA base oxidation, DNA–protein cross-links, DNA gaps or breaks, and histone hydrolysis could potentially result from the interaction of Ni with DNA, histones, and/or the generation of reactive oxygen species via Ni redox chemistry or indirect effects (2).

Because Ni\textsubscript{2+} ions do not bind strongly to DNA in competition with Mg\textsuperscript{2+}, other biological complexes of Ni are implicated, and complexes formed with histone proteins are one likely target. Nuclear histones have structures consisting of globular C-terminal domains and random-coil N-terminal domains (42–45). The N-terminal tail regions are involved in internucleosome contacts and are the site of posttranslational modification via acetylation, which has been correlated with gene expression. Inspection of the amino acid sequence of histones (46) does not reveal a large number of potential metal-binding sites because of the general lack of amino acid residues with side chains bearing Lewis bases at physiological pH. Histone H2A contains the –T\textsubscript{120}ESHHK– sequence near the C-terminus (47). This site has a glutamic acid and two histidine residues that could engage in metal chelation. A second potentially chelating site composed of cysteine and histidine residues has been identified in the C-terminal region of histone H3: –C\textsubscript{110}AIH– (48, 49). Last, a site near the N-terminus of histone H4, –A\textsubscript{18}KRHRK–, has also been proposed as a site for Ni binding largely because of the inhibitory effects of Ni on the acetylation of lysine residues near the His residue (50).

The natural biochemistry of Ni suggests that only the H3 site can possibly give rise to Ni(III/II) redox chemistry, because it is the only site with a potential cysteinate ligand that might lower the potential of this redox couple. Even this site is not likely to stabilize Ni(III/II) redox chemistry because there is only a single cysteine present. This site is also unlikely to inhibit lysine acetylation given that the location of the H3 site is in the C-terminal region. Further, the structural data regarding histones (42–45) indicate that the site is not very accessible to solvents or metals. Even though metal-centered redox chemistry can be ruled out for all but the H3 site, any of the sites could potentially activate DNA or histone proteins toward reaction with O\textsubscript{2}, as has been suggested for the Ni site in acireductone dioxygenase (51). Each of these sites presents a unique set of ligands that could readily be distinguished by XAS provided that a homogeneous sample of a single Ni histone complex is formed. However, because of its location in the N-terminal domain, only the H4 site is likely to give rise to the changes in histone acetylation and DNA methylation that have been observed in response to Ni exposure.

The metal-binding properties of synthetic peptides that contain the –A\textsubscript{18}KRHRK– H4 target Ni binding site have been studied (52). Stability constants determined for the blocked peptide Ac–AKRHRK–Am show that >80% of Ni present in a solution at pH = 9 is bound in a single complex in solution. This complex is proposed to lead to a planar, four-coordinate, low-spin Ni tetra-aza complex formed via coordination of one His imidazole and three amide N-donor atoms.

The XAS spectrum of the Ni complex of the similar blocked peptide Ac–SGRGKKGKGLGKGGAKRRHKVL–Am, containing 22 residues beginning with the N-terminus and containing the H4 His\textsubscript{18} Ni-binding site, prepared at pH = 9, is summarized in Figure 2 (53). The Ni K-edge XANES spectrum reveals only a weak peak assigned to a 1s → 3d electronic transition [peak area = 2.6(5) \times 10\textsuperscript{-2} eV] and does not display a peak associated with a 1s → 4p, electronic transition that is diagnostic for a planar, four-coordinate geometry. Thus, the Ni complex that is formed from this peptide under these conditions is six coordinate. EXAFS analysis shows that Ni has six O- or N-donor atoms with an average Ni–O/N distance of 2.06(2) Å. The structure is consistent with four ligands being derived from the peptide and two originating from water. Multiple scattering analysis of EXAFS arising from atoms in the second and third coordination sphere of Ni (6) confirm that one His ligand is bound to Ni (second coordination sphere Ni–C distances averaging 3.12(5) Å, third coordination sphere Ni–O/N distances at an average of 4.26(5) Å).

Future studies are planned comparing the structure of Ni bound to histone H4 with those of this model and expectations based on other possible binding sites.
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