Molecular Mechanisms in Nickel Carcinogenesis: Modeling Ni(II) Binding Site in Histone H4

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Ni(II) compounds are well known as human carcinogens, though the molecular events which are responsible for this are not yet fully understood. It has been proposed that the binding of N(II) ions within the cell nucleus is a crucial element in the mechanism of carcinogenesis. The most abundant proteins in the cell nucleus are histones, and this makes them the prime candidates for this role. This article is a review of our recent studies of histone H4 models of Ni(II) binding. We analyzed the sequence of the N-terminal tail of the histone H4, Ac-SGRGKGGKGLGKGG AKRH$_{18}$RKVL-Am, for Ni(II) binding. This site has been proposed mainly because of the potent inhibitory effect of Ni(II) on the acetylation of lysine residues near the histidine H$_{18}$, and also because of the accessibility of the H4 tail in the histone octamer. Combined potentiometric and spectroscopic studies showed that the histidine 18 acted as an anchoring binding site for metal ions in the peptide investigated. Comparison with the results for Cu(II) binding are also reported. The results allowed us to propose that the binding of Ni(II) is able to promote a secondary structure with organized side-chain orientation on the N-terminal tail of histone H4. ***Key words:*** **nickel, histone H4, carcinogenesis.** *Environ Health Perspect* **110(suppl 5):719–723 (2002).** *http://ehpnet1.niehs.nih.gov/docs/2002/suppl-5/719-723zoroddu/abstract.html*

Nickel compounds are established human carcinogens according to International Agency for Research on Cancer (*1*). They are widely distributed in modern industries. Several industrial processes, e.g., nickel mining and refining, electroplating, the production of long-lasting nickel-cadmium batteries, the combustion of fossil fuels, and the incineration of nickel-containing solid waste, are responsible for the production of nickel-containing aerosols in the workplace and in the surrounding environments.

Because of the widespread use of these agents, workers in these facilities are at risk for occupational exposure. In addition, the release of nickel into the environment represents a potential risk for nonoccupational exposure. The average daily exposure to nickel by inhalation has been estimated to be 0.2 and 0.4 μg for rural and urban dwellers, respectively (*2,3*). Welders working with stainless steel are another professional group with an elevated risk for nickel-related carcinogenesis.

Inhalation is the main route for human exposure to nickel compounds, and epidemiologic studies have demonstrated a correlation between the incidence of respiratory (lung and nasal) cancer and worksite exposure to nickel. The inhalation of nickel-containing dusts and aerosols poses the major hazard present in nickel mines and refineries. The main target areas are those in the respiratory system, depending on the size of nickel-containing dust particles. The coarsest particles are deposited in the nose, mouth, and larynx, whereas the finer grains can reach the bronchial tree (*4,5*).

The relative carcinogenic activity of nickel compounds is related to their water solubility (*6*). Water-soluble nickel salts ($NiCl_2$, $NiSO_4$) are considered less carcinogenic than water-insoluble compounds, such as NiS, Ni_3S_2, and NiO, because the uptake of soluble nickel compounds is poor. The soluble nickel compounds dissolve in the mucus covering the airways, and the resulting ionic or complexed nickel is removed by the ciliary transport mechanism. The potent carcinogenic activity exhibited by crystalline nickel is due to the ability of the Ni_3S_2 particles to enter cancer target cells by phagocytosis (*7*).

Phagocytosis of particles, by either macrophages or epithelial cells, causes buildup of very high levels of nickel inside the cells after its intracellular slow dissolution (Ni_3S_2 is dissolved by oxidation), catalyzed by the acidic pH of endocytic vacuoles. A continuous source of Ni(II) ions is provided in this manner. Eventually the cells undergo neoplastic transformation, and cancer develops in a process that may take many years. The carcinogenic potency of nickel compounds is consistently related to the ability of Ni(II) to access chromatin and cause multiple types of cellular-nuclear damage via direct or indirect mechanisms, including *a*) promutagenic DNA damage, that is, oxidative damage to nucleobases as well as DNA strand breaks (*8,9*); *b*) impairment of DNA repair mechanisms by nickel (*10,11*); and *c*) epigenetic effects in chromatin, such as chromatin condensation and inhibition of histone H4 acetylation (*12–15*).

Depending on the condition, it is possible that dual mechanisms, both genotoxic (initiation) and epigenetic (promotion and progression), can be operating (*16*). To understand how damage affects different nuclear components, we need to consider briefly the structure of the cell nucleus. The most fundamental building block of the cell nucleus is called the nucleosome. It is composed of an octameric assembly of proteins called the core histones. The core histone proteins and the nucleosomes they form with DNA constitute the eukaryotic chromatin. The core histone proteins (H3, H4, H2A, and H2B), together with the linker histone H1, package eukaryotic DNA into repeating nucleosomal units that are folded into higher-order chromatin fibers. Once considered static nonparticipating structural elements, it is now clear that histones are integral and dynamic components of the machinery responsible for regulating gene transcription.

DNA is held on the surface of the protein assembly by a trace of positively charged Arg and Lys side chains and also by whiskers of extended N-termini of histones (*17*). Histone N-terminal tails are central to the processes that modulate nucleosome structure and function. An extensive literature documents an elaborate collection of posttranslational modifications, including acetylation, that take place on the N-terminal tail domains of histones. Of these modifications, acetylation and deacetylation have generated much interest because gene activity is correlated with histone acetylation (*18*).

Acetylation is an energy-intensive, dynamic phenomenon, of which the steady-state balance is mediated by the opposing activities of histone acetyltransferase and deacetylase enzyme systems. The acetylation reaction involves the transfer of an acetyl group from acetyl-coenzyme A onto the amino group of specific lysine residues present in the amino-terminal tails of each of the core histones, resulting in the neutralization of a single positive charge (*19*).

The continued interest in acetylation-related phenomena undoubtedly stems from the fact that the core histones are among the

This article is part of the monograph *Molecular Mechanisms of Metal Toxicity and Carcinogenicity.*

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Received 21 March 2002; accepted 4 June 2002.

most conserved protein in eukaryotes, and the specific lysines that undergo acetylation are absolutely conserved in most cases. This implies that the N-termini do not function simply by interacting nonspecifically with the DNA components of chromatin, because such a mechanism would not require such an extraordinary degree of sequence conservation.

N-termini are engaged primarily in protein–protein interactions, which cause N-termini to adopt specific secondary structure, and recent evidence suggests that the function of acetylation may be to disrupt these secondary structure motifs (*19,20*). Long-standing models have suggested that histone acetylation may alter chromatin structure by influencing histone–DNA, histone–histone, and intranucleosome contacts; converging biochemical and genetic evidence suggests functions in several chromatin-based processes. This may allow the termini to be displaced from the nucleosome, causing the nucleosome to unfold, and increasing access to transcription factors (*21,22*). It could cause an opening of these particles, increasing the accessibility of DNA for regulatory factors, and could provide a mechanism for the establishment of an open, active chromatin structure. This does not exclude an additional role for histone tails in chromatin fiber compaction.

A more recent hypothesis proposes that a histone language (read by other proteins or protein modules) may be encoded on these tail domains. Covalent modification like histone acetylation could provide such a histone code showing that every amino acid in histone tails has specific meaning and is part of the vocabulary of the overall code (*23*). This suggests that any alteration in tail structure could be crucial. These tails, which protrude from the surface of the chromatin polymer and are protease sensitive, comprise about 25–30% of the mass of individual histones, thus providing an exposed surface for potential interactions.

Although the molecular mechanisms involved in nickel toxicity and carcinogenicity are not fully understood, several studies demonstrate that binding of nickel inside the cells is the factor leading to all the cellular pathways hypothesized to explain nickel activity. Numerous studies point to the cell nucleus as the site of nickel attack (*12–14*), thus, the identification of cellular-nuclear binding sites for nickel and the basic chemical activities resulting from the binding modes are objectives essential to understanding the mechanisms involved.

Following this line, DNA polymers bind Ni(II) only weakly *in vitro* (*24*). The binding of Ni(III) to DNA is ionic and involves phosphate oxygens. Such Ni(II) can be easily displaced by other divalent cations, especially by physiological DNA counterion Mg (II). In addition, phospholipids of cellular membranes do not provide high-affinity binding sites for Ni(II). This leaves the proteins and low-molecular-weight chelators (e.g., amino acids and peptides) as the most likely nickel-binding molecules. Of all macromolecular components of the cell nucleus except DNA itself, histones are by far the most abundant among nuclear proteins in somatic cells, reaching a formal concentration of 3 mM (*25*). Therefore, by the law of mass action, if Ni(II) binding sites of even moderate affinity could be found in the histones, they might be able to compete for Ni(II) with higher affinity sites in other but less-abundant nuclear proteins, or with low-molecular-weight chelators.

Indication for an existence of such a site on core histones is provided by the fact that nucleohistones largely enhance 8-oxo-7,8-dihydro-2′-deoxyguanosine formation (the major product of reactive oxygen species attack in genomic DNA). In addition, unlike pure DNA, exposure of chromatin (nucleohistones) to ambient oxygen in the presence of Ni(II) added as a noncomplexed salt also results in an increased DNA base oxidation (*26*). In fact Ni(II), especially in the form of nickel complexed with certain natural ligands, enhances the oxidation damage to DNA bases (*27*). Thus, modulation of the damage by the protein component of chromatin clearly indicates complexation of nickel, most likely by the histones. All these facts make nuclear proteins the primary targets for Ni(II) ions.

The detection and the structural and mechanistic description of specific Ni(II) sites in histones could provide a molecular basis for better understanding the mechanisms underlying Ni(II)-induced carcinogenesis. This simple notion was the starting point for our proposal to analyze and investigate histones for Ni(II) binding. We reviewed the published amino acid sequences of the histones (*28*), as well as the structural data on histone octamer, to identify possible Ni(II) binding motifs and subsequently to investigate the formation and properties of Ni(II) complexes with those motifs.

Ni(II) is a borderline metal ion capable of forming stable complexes with both hard (oxygen) and soft (nitrogen, sulfur) donors. Available information on the binding modes of Ni(II) to proteins and data for nickel-peptide complexes indicate, however, that imidazole of histidine and thiol of cysteine should be thermodynamically preferred by Ni(II) among the donor groups provided by protein-building amino acids (*29,30*). Carboxyl groups of aspartate and glutamate can play only secondary roles in binding. There are two general modes of interaction of transition metal ions, including Ni(II), with peptides and proteins. The peptide mode involves binding to the terminal groups and deprotonated amide bonds of the peptide backbone. It occurs in peptides containing noncoordinating side chain in the amino terminal residues. The protein mode uses only the reactive side-chain groups; histidine imidazole and cysteine thiol are most effective in nickel binding. When histidine (or cysteine) appears along the peptide chain, they facilitate peptide deprotonation by providing an anchoring binding site for a metal ion. The search for potential nickel-binding sites in the

Figure 1. Sequences of predominant form of human histones. Histidine and cysteine residues are underlined, and the tail of histone H4 is evident.

histones should therefore be focused on terminal sequences histidine and cysteine.

Representative examples of the sequences of predominant forms of human histones H1, H2A and H2B, H3, and H4 are presented in Figure 1 (*9*). As one can clearly see, the linker histone H1 does not contain any His or Cys residues, and contains only a few carboxylates. Inspection of the available histone sequences revealed several histidine and cysteine residues in H2, H3, and H4. However, examination of the X-ray crystal structure of the nucleosome core particle reveals that histidine and cysteine residues in H2 and H3 are located inside the molecule in the protein interior. A histidine (His_{18} from the N-terminal) can be seen in the histone H4 N-terminal tail that extends from the core, where it is accessible (and can be posttranslationally modified by acetylation). Histone H4 is one of the most conserved proteins in nature, even for the amino terminal region (residues 1–22, 23). This region features three repetitions of the sequence gly–lys–gly and the unusual string of five basic residues -KRHRK- (Figure 2), which may serve as a binding locus for selectively binding nucleic acid.

We found previously that nickel is a potent inhibitor of histone H4 acetylation in yeast and in mammalian cells (*15*). Interestingly, an anchoring binding site for metal ions, a histidine, is close to sites for posttranslational modification involved in nickel and, to a lesser extent, in copper toxicity.

All this information points to the H4 tail as a candidate for an Ni(II) binding site in the histone octamer. Because of its structural position, this terminal part could be accessible for metal binding. For this reason we analyzed, for Ni(II) and Cu(II) binding, the sequence of the N-terminal tail of the histone H4, the 22-amino acid peptide Ac-SGRGKGGKGLG KGGAKRHRKVL-Am. The C-terminus was blocked by amidation to make the tail a more relevant model of the entire protein. In addition, the 7- and 11-amino acid peptides Ac-AK(Ac)RHRK(Ac)V-Am and Ac-GK(Ac)GGAK(Ac)RHRK(Ac)V-Am, where all side chains of lysines were blocked by acetylation, were studied to verify whether the acetylation of lysines affects coordination behavior. The binding study was also extended to Cu(II) to analyze if differences in biological activity can result in differences in coordination behavior.

Material and Methods

Potentiometric and Spectroscopic Measurements

Stability constants for protons Ni(II) and Cu(II) complexes were calculated from titration curves carried out at 25°C using a total volume of 1.5 cm^3. NaOH was added from a 0.250 cm^3 micrometer syringe calibrated by both weight titration and the titration of standard materials. Metal ion concentration was 1.5×10^{-3} mol dm^{-3}, and the metal-to-ligand molar ratio was 1:1.1. pH meter titrations were performed at 25°C in 0.10 mol dm^{-3} KNO_3 on a Molspin pH meter system (Molspin Ltd., Newcastle upon Tyne, UK) using a microcombined glass/calomel electrode calibrated in hydrogen ion concentrations using HNO_3 (*31*). The SUPERQUAD computer program (Superquad for the Calculations and Metal-Complex Stability Constants, Version 5.2, L.D. Pettit, Academic Software, Sourby Farm, Timble Otley Yorks, UK) was used for stability constant calculations (*32*). Standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They are, however, a good indication of the importance of a particular species in the equilibrium.

It was not possible to perform the potentiometric measurements for Ac-GK(Ac)GGAK(Ac)RHRK(Ac)V-Am because of the hydrolysis with raising the pH. In this case only spectroscopic measurements were carried out.

Solutions for spectroscopic measurements were of similar concentrations to those used in the potentiometric studies. Absorption spectra were recorded on a Beckman DU 650 spectrophotometer Beckman Instruments Inc., Fullerton, CA, USA). Circular dicroism (CD) spectra were recorded on a JASCO J 600 spectropolarimeter (JASCO, Spectroscopic Co., Hiroshima, Japan) in the 750–250 nm range. The values of $\Delta\varepsilon$ (i.e., $\varepsilon_l - \varepsilon_r$) and ε were calculated at the maximum concentration of the particular species obtained from the potentiometric data.

Results and Discussion

The 22-amino acid sequence can be considered an H_6L ligand, where the deprotonation involves the histidine residue (pK_a = 5.99) and the five side chains of the lysine residues (pK_a = 11.79, 10.75, 10.08, 10.03, and 9.20). The 7- and 11-amino acid peptides [Ac-AK(Ac)RHRK(Ac)V-Am and Ac-GKGGAK(Ac)RHRK(Ac)V-Am] are an HL ligand where the deprotonation involves the histidine residue.

The 22-amino acid peptide Ac-SGRGKGGKGLGKGGAKRHRKVL-Am binds Ni(II) and Cu(II) through the imidazole nitrogen, starting at pH 7 and at pH 3.5, respectively, and resulting in 1N complexes. When the pH was raised, both metal ions deprotonated successive peptide nitrogens, forming M–N^- bonds, until MH_2L species (4N complexes) were formed above pH 8 for Ni(II) and pH 6 for Cu(II). The formation of the stable five-member chelate rings by consecutive nitrogens is the driving force for the coordination process (Figure 3).

The logK* value is a good measure of the stability of the complex species, describing competition of metal ions with protons for peptide binding [$\log K^* = \log\beta\ MH_{n-j}L - \log\beta\ H_nL$ for the reaction $M(II) + H_nL \blacklozenge MH_{n-j}L + jH^+$]. The stability of the 4N complex of Cu(II) with the 22-amino acid peptide is higher (logK* = –21.79) compared with that obtained for shorter fragments and is comparable to that of 14-amino acid peptide Ac-TRSRSHTSEGTRSR-Am, Cap43 (*33*). The stability of the 4N complex of Ni(II) with the 22-amino acid peptide is

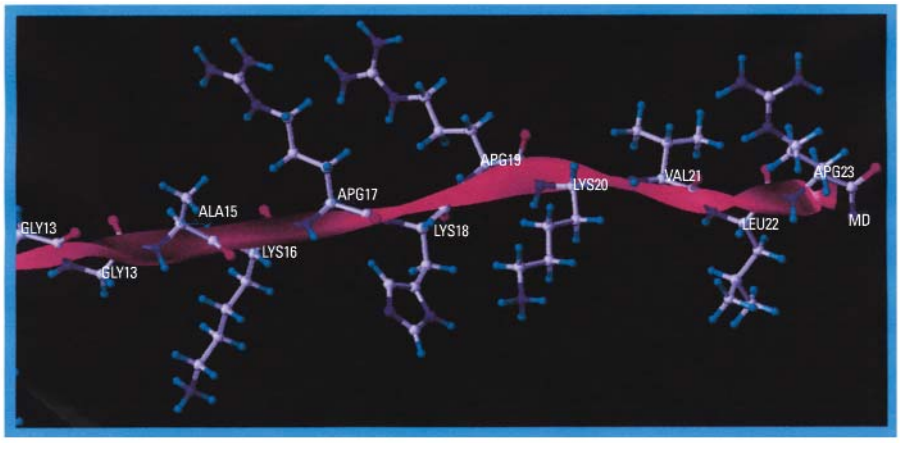

Figure 2. AKRHRK sequence. The ribbon represents the backbone of the peptide.

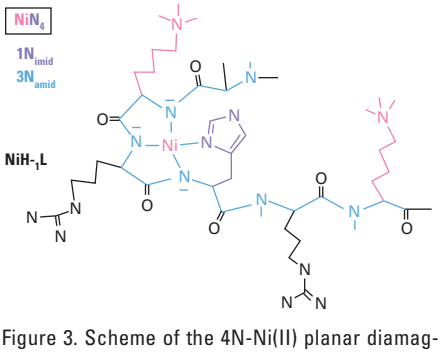

Figure 3. Scheme of the 4N-Ni(II) planar diamagnetic species.

higher (logK* = –28.67) or of similar stability compared with that obtained for shorter N- and C-blocked peptides containing the histidine residue (*34*). Coordination behavior was not significantly affected by the acetylation of lysines.

The pK_a values of N(3) imidazole nitrogen of the histidine residue of the tail and of the models investigated are of the same order of magnitude (ranging from 5.99 to 6.13). They are more acidic than other simple peptides, about one order of magnitude more acidic than histidine in Boc-AGGH, Ac-GGGH, and Ac-GGH (7.19, 7.21, and 7.18, respectively), where glycine instead of arginine or lysine residues are close to the anchoring site for metal ions. It is interesting to note that the pK_a (NH_{im}^+) values for our motifs are of the same order of magnitude as that of N-terminal free peptides and where electron-withdrawing groups substitute a hydrogen at the pyrrolic nitrogen (*35*). In this case the less basicity is due both to the electrostatic effect of the positive charge from NH_3^+ terminus and to the inductive effect of the substituting group. In our case the minor basicity can be associated with the electrostatic effect from the positively charged side chains of R and K, or R, in the peptide ligands. The log K* values for the formation at N(3) of imidazole and of 1N complexes of acetylated peptides are linearly related to the pK_a (NH_{im}^+) in the free peptides (*36*).

It is to be expected that the labilizing effect of the metal ions on the peptide protons will be strongly influenced by the extent of electron donation by the group acting as the initial coordination locus. Hence, a lower pK_a (NH_{im}^+) value will be associated with a lower degree of electron donation to the metal ion, which will be reflected in a lower value for pK_a amide. Therefore, the lower the basicity of the N(3), the more simple the deprotonation, promoted by metal ions, of amide nitrogens, resulting in an enhancement of π-electron contribution to the metal amide nitrogen bond compared with Boc-AGGH or other simple peptides (*37*).

Ultraviolet-visible (UV-Vis) spectra are in agreement with the results from potentiometric measurements. Figure 4 shows the UV-Vis spectrum, obtained with changing the pH, for the mixture of Ni(II) ions with the Ac-GK(Ac)GGAK(Ac)RHRK(Ac)V-Am fragment. The λ_{max} value at 439 nm (ε = 104 $dm^3\ mol^{-1}cm^{-1}$) with a shoulder at 494 nm is characteristic of a planar coordination of Ni(II) ions in a 4N chromophore (*38*).

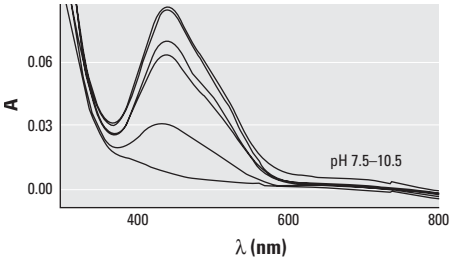

Figure 4. UV-Vis spectrum of the Ni(II) Ac-GK(Ac)GGAK(Ac)RHRK(Ac)V-Am species with change in pH.

The impact of the secondary peptide structure on the complex stability cannot be excluded (*39*). The CD spectra of the fully protonated and deprotonated form of the tail are qualitatively the same, resembling those of unordered peptides. It is interesting to note that on coordination of the tail in a 4N complex with Ni(II), unlike motifs with protected lysines and unlike Cu(II), there was a change in the spectrum with a value of Δε about 5 mol $cm^{-1}dm^{-3}$ in the region (220–230 nm) dominated by the peptide carbonyl chromophore (spectrum not shown). This behavior suggests a possible bent structure with organized side-chain orientation promoted by Ni(II) (*39*).

Although the complexation with Ni(II) at a physiological pH under our experimental conditions is not very effective, the formation of a rigid square-planar complex may result, somewhat paradoxically, in a higher specificity of Ni(II) to produce a particular conformation of the peptide (*40*). The presence of positively charged residues close to the metal-binding site in the H4 tail can result in a site-selectivity association of the Ni(II) complexed tail with the negatively charged DNA backbone (*41*). In addition the hydrophobic environment in the entire protein is expected to enhance metal-binding capabilities because of multiple nonbonding interactions available there, as reported in the literature (*42–44*).

In conclusion histidine-18 residue can be a primary binding site for Ni(II) ions in the H4 tail. The coordination ability of the entire tail toward Ni(II) is similar to that found for the hexapeptide fragment AKRHRK (*45*), but the conformation behavior is dependent on the chain length and on the metal ion. In fact, Ni(II) coordination to the 22-amino acid peptide induces organized side-chain orientation unlike Cu(II) and unlike motifs with protected lysines. The data obtained with Ni(II) and Cu(II) metal ions point out an interesting aspect, as they seem to show that the sequence of the H4 amino-terminal tail can adopt different conformations, depending on the metal ion. All these facts may be physiologically relevant to the mechanism of nickel-induced carcinogenicity.

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