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# Nickel binding sites in histone proteins


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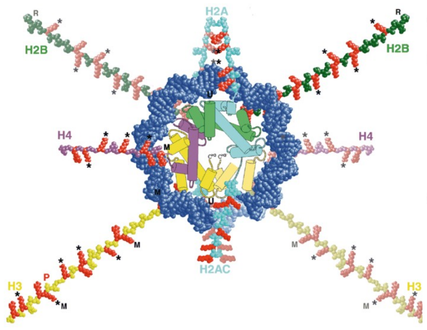
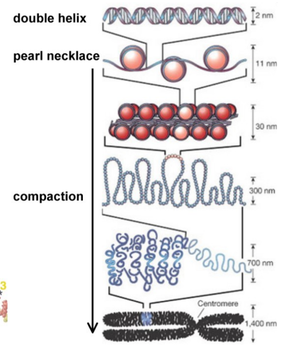
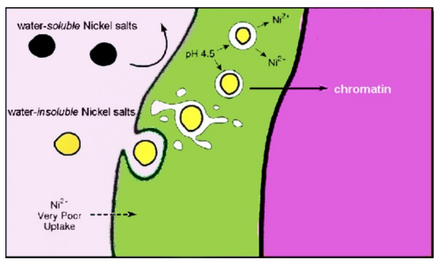
## Introduction

Nickel compounds are well known as human carcinogens [1], though the molecular events that are responsible for this are not well understood.
It has been proposed that a crucial element in the mechanism of carcinogenesis is the binding of Ni(II) ions within the cell nucleus. It is known that DNA polymer binds Ni(II) only weakly, leaving the proteins of the cell nucleus as the likely Ni(II) targets [2-7]. Being histone proteins the most abundant among them, they can be considered the primary sites for nickel binding. Here we describe the interactions of nickel with histone H4, core tetramer $(H3\text{-}H4)_2$ and several peptide fragments which have been selected as the candidates for specific binding sites in the histone octamer. The results allowed us to propose several mechanisms of nickel induced damage resulting from metal coordination, including structural changes of histone proteins, as well as nucleobase oxidation and sequence-specific histone hydrolysis.
The aim of the present work is to provide a comprehensive overview of literature dealing with nickel coordination to histone proteins and its link with nickel involvement in toxicity and carcinogenicity.




Histones H4, H2A, H2B and H3 are nuclear protein which package and order the DNA into structural units called nucleosomes. Each histone contains a carboxy-terminal, highly helical globular domain that forms the interior core of the nucleosome particle. The remaining amino-terminal portion of the core histone proteins contains a flexible and highly basic tail region and is the site for several types of post-translational modification, including methylation, phosphorylation, ubiquitylation and acetylation. Such post-translational modifications have long been correlated with various nuclear activities, including gene replication and transcription.




Although nickel seems to be essential for some bacteria and plants, it has been evaluated as a carcinogenic agent by IARC, after a combination of epidemiological evidence in human, in animals and in cultured cells. The relative carcinogenic activity of Ni compounds has been consistently related to their water solubility and to the ability of insoluble nickel compounds to access chromatin. 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 carcinogenic activity exhibited by crystalline nickel is due to the ability of the insiluble particles to enter cells by phagocytosis.
The endocytic vescicles formed ultimately releasing large quantities of Ni ions into the cytoplasm

**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:**

- ❖ promutagenic DNA damage
- ❖ oxidative damage to nucleobases
- ❖ DNA strand breaks
- ❖ impairment of DNA repair mechanisms
- ❖ epigenetic effect in chromatin derangement of transcription regulation, inhibition of H4 acetylation
- ❖ genetic effect, enhance expression of transcription factors

The nuclear proteins, and in particular the most abundant amongst them, the histones, are able to compete for metal ions with even higher affinity for metal binding sites in other less abundant nuclear proteins or smaller molecules.
Data for nickel peptide complexes indicate that imidazole nitrogen of Histidine and thiol sulfur of Cysteine should be preferred by Ni(II) amongst the donor groups of amino acids.
So, we examined the amino acid sequences of histones in order to identify potential Nickel binding motifs:

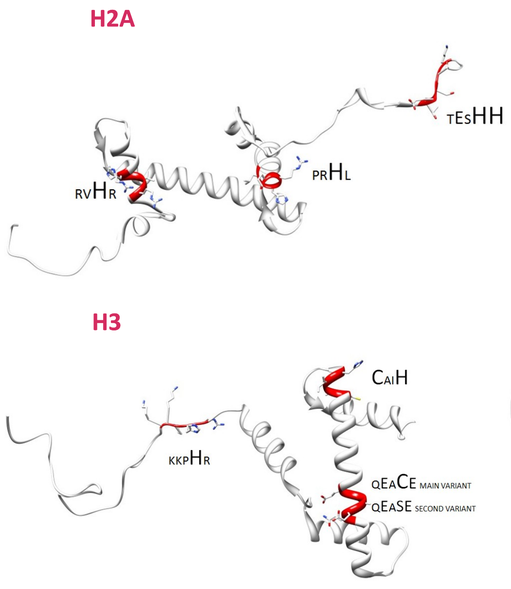
**Histone H2A**
SGRGKQGGKARAKAKTRSSRAGLQFPVGRVHRLLRKGNYAERVGAG
APVYLAAVLEYLTAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLL
GKVTIAQGGVLPNIQAVLLPKKTESHHKAKGK

**Histone H2B**
PEPAKSAPAPKKGSKKAVTKAQKKDGKKRKRSRKESYSIYVYKVLKQVH
PDTGISSKAMGIMNSFVNDIFERIAGEASRLAHYNKRSTITSREIQTAV
RLLLPGELAKHAVSEGTKAVTKYTSSK

**Histone H3**
ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVA
LREIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACE
AYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGEA

**Histone H4**
SGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISG
LIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQGRTL
YGFGG

Sequences of predominant form of human histones. Histidine and cysteine residues are highlighted in purple and yellow color respectively.




Potential binding sites for Ni(II) ions in histone H2A, H2B, H3 and H4. The potential Nickel binding sequences are indicated and red highlighted. Histone structures were extracted from the X-Ray Structure of the Nucleosome Core Particle, NCP147 (PDB 1KX5).

## References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## Nickel bindind to Histones

Inspection of the available histone sequences revealed several histidine and cysteine residues in H2, H3, and H4 proteins. Examination of the X-ray crystal structure of the nucleosome core particle, however, reveals that some histidine and cysteine residues are mainly located inside the molecule in the protein interior and, for this reason, less accessible for metal binding.
Here we report, by way of an example, the study of the interaction of Ni(II) with the binding site located in the N-terminal tail of Histone H4: **AKRHRK.**
Because the study of the interaction of metal ions with a complex system as the histone octamer is a very difficult task, the authors began the investigations with on different peptide fragments.

## Ni(II) binding to Histone H4

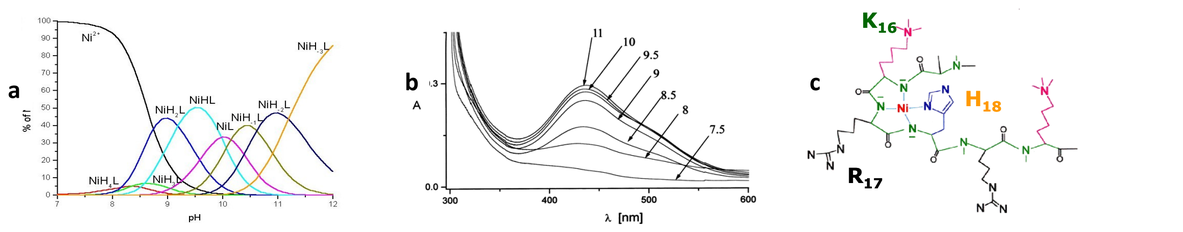
Nickel is a potent suppressor of histone H4 acetylation, in both yeast and mammalian cells, and this lead to transcription errors and subsequent DNA modifications. It has preference towards Lys-12 (K12) in the N-terminal histone H4 domain, in which the sites of acetylation $K_5$, $K_8$, $K_{12}$, $K_{16}$ are clustered .
A histidine $His_{18}$ is close to the site of acetylation, and is located in the N-terminal tail that extends out from of the core, where it can be more accessible for metal coordination.
The **–AKR$H_{18}$RK** motif has been studied as a minimal model of the entire H4 Histone, and subsequently the length of the sequences has been extended until the whole protein.

| | |
|---|---|
| **6aa** | Ac-AKR$H_{18}$RK-Am |
| **7aa** | Ac-AK$^{(Ac)}$R$H_{18}$RK$^{(Ac)}$V-Am |
| **11aa** | Ac-GK$^{(Ac)}$GGAK$^{(Ac)}$R$H_{18}$RK$^{(Ac)}$VL-Am |
| **22aa** | Ac-SGRGKGGKGLGKGGAKR$H_{18}$RKVL-Am |
| **30aa** | Ac-SGRGKGGKGLGKGGAKR$H_{18}$RKVLDNIQGIT-Am |

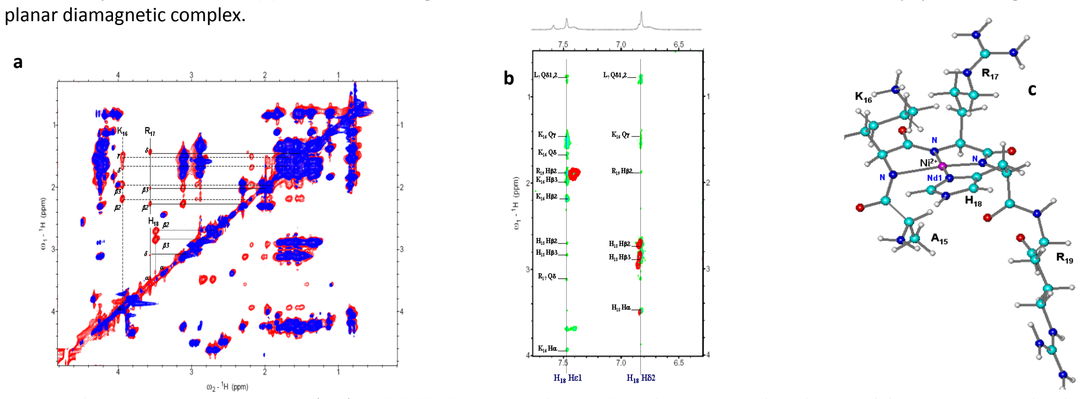
**H4 protein**
SGRGKGGKGLGKGGAKR$H_{18}$RKVLDNIQGITKPAIRRLARRGGVKRISGLIYEET
RGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFGG

NMR, UV-Visible and CD spectroscopies, together with potentiometric titration, have been used to investigate the binding of Ni(II) with several peptide models of the histone H4 tail.
Short models of the N-terminal tail: ***6aa, 7aa, 11aa and 22aa***
Full N-terminal tail: ***30aa***
Whole histone H4: ***103aa***
Multi-dimensional NMR techniques, such as $^1$H-$^1$H TOCSY, $^1$H-$^{13}$C and $^1$H-$^1$H ROESY, were performed in order to identify the amino acids involved in Ni(II) coordination, the conformational changes experienced by the peptides upon nickel binding, and to determine the structural model of the metal complex formed.

By combined potentiometric and spectroscopic studies it resulted that histidine $H_{18}$ acts as an anchoring site for nickel ions .
The peptide N-terminal tail of Histone H4 binds Ni(II) through the imidazole nitrogen, starting at pH 7.
By increasing the pH, Ni(II) is able to deprotonate successive peptide nitrogens forming M-N$^-$ bonds, until a 4N complex is formed.

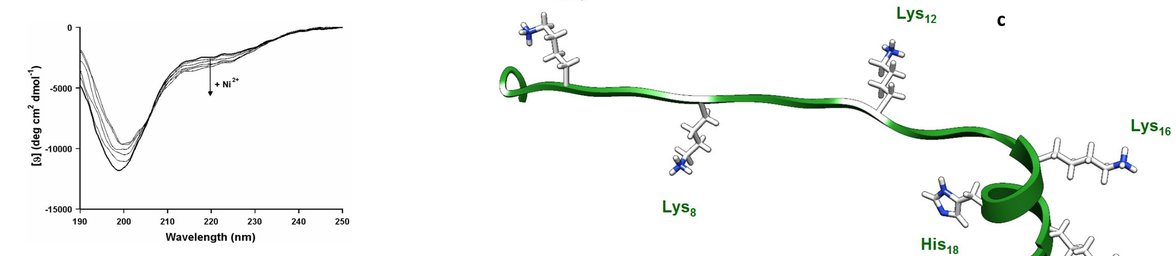



a) Species distribution curves for Ni(II) complexes of Ac-SGRGKGGKGLGKGGAKRHRKVL-Am, 1:1 molar ratio
b) UV-Vis spectra of Ni(II) complexes with histone H4 tail, in a 1:1 molar ratio, at increasing pH values.
c) Schematic representation of Ni(II) ion bound through the imidazole nitrogen and deprotonated successive peptide nitrogens in a 4N-Ni(II) planar diamagnetic complex.




a) Overlaid of the aliphatic region in the $^1$H–$^1$H NMR TOCSY spectra for the 30aa free peptide (blue) and Ni(II) bound peptide (red) at a 1:0.8 peptide-to-nickel molar ratio. New resonances due to Ni-binding have been labeled.
b) Overlaid of the aromatic region in the $^1$H–$^1$H NMR NOESY (green) and TOCSY (red) spectra of Ni(II) bound 30aa peptide at a 1:0.8 peptide-to-nickel molar ratio.
c) Three-dimensional structure of peptide-Ni(II) complex, obtained from NMR data calculations.

From a circular dicroism study we found that nickel is able to induce a secondary structure in the protein:
**it induces an increase in the α-helical conformation of the non-acetylated histone H4.**




a) CD spectra of a solution containing H4 protein and Ni(II) ions at pH 8.7 (5 mM tetraborate/HCl buffer) with increasing nickel concentrations (the thick line refers to metal free histone H4).
b) Aromatic region of 600 MHz 1D NMR spectra of H4 histone protein with increasing amounts of Ni(II) from 0 eq. (free peptide) to 2 eq.
c) Molecular-mechanics optimized structure of the helical conformation adopted by the non-acetylated H4 N-terminal tail in the presence of Ni(II) ions (only lysine and histidine residues are shown).

## Conclusions

Spectroscopic data confirmed the square-planar coordination adopted by nickel, and NMR experiments identified the donors atoms as the N from imidazol of $His_{18}$ and the amidic N from $His_{18}$, $Arg_{17}$ and $Lys_{16}$ of the backbone of the peptide.
Using the recorded NOEs, we were able to calculate the structure of the complex which shows that the side chain of $Lys_{16}$ and $Arg_{17}$ are blocked above the coordination plane. The conformation of $Lys_{16}$ side chain can be crucial in the enzyme recognition processes, i.e. in the acetylation mechanism promoted by the acetyltransferase enzyme HAT.
From a circular dicroism study we found that nickel is able to induce a secondary structure in the protein: it induces an increase in the α-helical conformation of the non-acetylated histone H4.
Any conformational change of $Lys_{16}$ and the shortening of the distance between adjacent amino acids, caused by the translation from an extended to an helical conformation upon nickel binding, could disrupt the histone recognition motif; this impairs the activity of HAT leading to transcription errors and subsequent DNA modifications.