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NMR study of Nickel binding to N-tail of Histone H4

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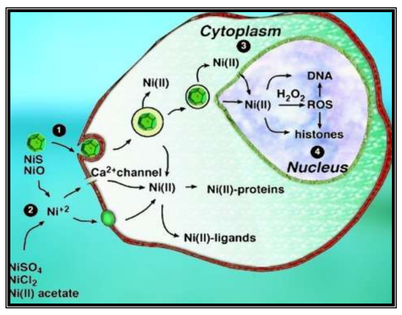
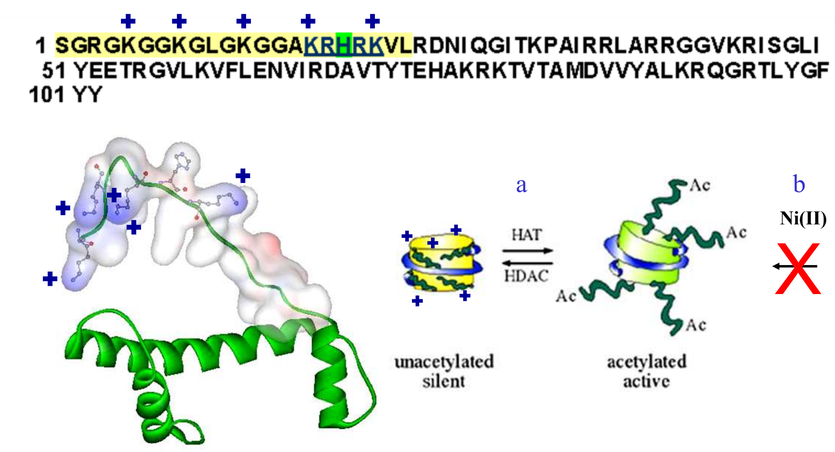
INTRODUCTION

Nickel has been shown to be an essential trace element involved in the metabolism of several species of bacteria, archea, plant and may yet be found to play a role in the metabolism of higher organisms[1] . However, the carcinogenicity of certain nickel compounds has been confirmed by the combination of epidemiological evidence in humans and carcinogenesis bioassays in animals [2]. The molecular mechanisms of nickel-induced carcinogenesis include interactions of this metal with major chromatin components causing alterations in gene expression rather than by direct DNA damage. The nuclear proteins, and in particular the most abundant among 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. Phagocytosis of insoluble particles of NiS by either macrophages or epithelial cells causes buildup of very high levels of nickel inside the cells after its intracellular dissolution catalyzed by the acidic pH of endocytic vacuoles, thus providing a continuous source of Ni(II) ions [3].



Phagocytosis of Nickel salts particles

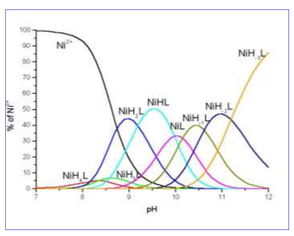
We have previously reported that nickel is a potent suppressor of histone H4 acetylation, in both yeast and mammalian cells [4-6]. It has preference towards Lys-12 (K12) in the N-terminal histone H4 domain, in which the sites of acetylation K5, K8, K12, K16 are clustered.
We investigated the issue of Ni(II) binding within the histone octamer. The interaction of this metal with the N-terminal tail, **Ac-SGRGKGGKGLGKGGAKRH$_{18}$RKVL-Am**, of Histone H4 was studied because of the potent inhibitory effect of Ni(II) on the acetylation of lysines residues near the histidine H_{18}, and also because of the accessibility of the H4 tail in the histone octamer.
The acetylation pattern and protein interactions of the N-termini of histone H4 in yeast telomeres were found to be crucial for the establishment of gene silencing.



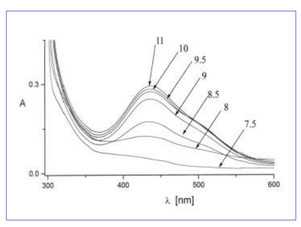
The blue color of the surface in the tail indicate the positive charge of lysines

in nucleosomes can be acetylated (Ac) by HATs or deacetylated by HDACs. The acetylation state influences the chromatin architecture
b) Inhibition in vivo of histone H4 acetylation by Ni(II)

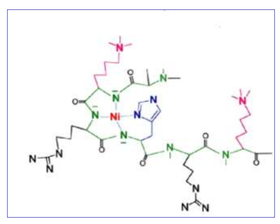
By combined potentiometric and spectroscopic studies we verified, that histidine H_{18} acted as an anchoring binding site for nickel ions [5-6].



Species distribution curves for Ni^{2+} complexes of Ac-SGRGKGGKGLGKGGAKRHRKVL-Am:Ni^{2+} molar ratio 1:1



UV-Vis Spectra of Ac-SGRGKGGKGLGKGGAKRHRKVL-Am:Ni^{2+} 1:1 with changing the pH



Schematic representation of Ni(II) ion bound through the imidazole nitrogen and deprotonated successive peptide nitrogens

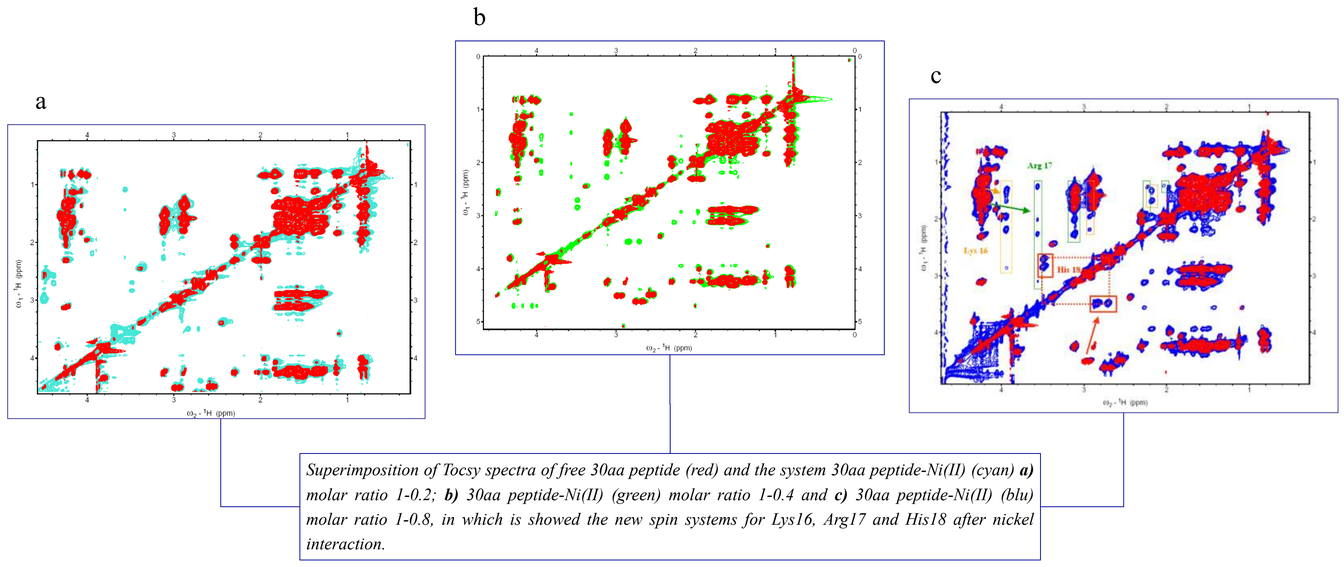
NMR STUDY

The coordination ability of Ni(II) to the N-terminal tail of Histone H4 were studied using NMR spectroscopy.

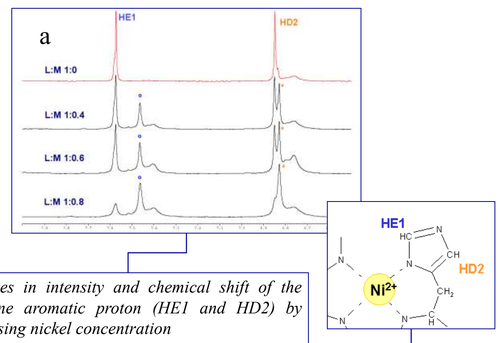
NMR Spectroscopy

NMR experiments were performed on a Bruker Advance 600 or 700 MHz spectrometers equipped with 5 mm TXI 1H-13 probe. Samples used for NMR experiments were 5 mM concentration and dissolved in 90% H2O/10% D2O solutions. All acquisition were performed at the temperature of 298 K. A series of 1D spectra of the free peptide was recorded at various pH values between 2.7 and 10.0 by step of 1.0 to provide a titration curve for imidazole protons. The sample pH was adjusted to the final pH by addition of 1 N NaOH or 1 N HCl. The titration experiments of Ni(II)-containing samples with ratios of 1:1 were performed at pH 8.7. Nuclear Overhauser enhancement spectroscopy (NOESY) with mixing times of 500 ms and Total Correlation Spectroscopy (TOCSY) with a mixing time of 50 ms were also performed. The combination of TOCSY and NOESY experiments was used to assign the spectra of both free and Ni(II)-bound peptide. Solvent suppression for 1D, TOCSY and NOESY experiments was achieved using WATERGATE pulse sequence or using excitation sculpting with gradients. All NMR data were processed using XWINNMR (Bruker Instruments) software on a Silicon Graphics Indigo workstation and analyzed using the Sparky 3.11 program

A comparison of the 1D, 2D ^{1}H homonuclear TOCSY and NOESY NMR spectra of 30aa H4 free peptide and of peptide-Ni(II) species was performed at pH= 8.7. This pH was chosen to approach maximum formation of the major planar diamagnetic species, as evidenced by potentiometric and spectroscopic measurements. The resonances belonging to the 30 residues of the free peptide were assigned on the basis of 1D NMR spectra and 2D ^{1}H homonuclear TOCSY and NOESY experiments. In the region between 6.6 and 8.5 ppm, only the aromatic resonances of histidine HE_1 and HD_2 at 7.595 and 6.862 ppm, respectively, were present. All the amide resonances were in a fast exchange with water at this pH and their resonances were lost. In the aliphatic region, the Hα of histidine appeared at 4.513 ppm. Its assignment was based on the analysis of the TOCSY spectrum, where a correlation between Hα histidine and its βH was visible ($H\beta_1$ 3.042 ppm $H\beta_2$ 2.970 ppm). The TOCSY and NOESY spectra also allowed the assignment of the entire spin system of every amino acid.
The binding mode of Ni(II) to the H4 sequence was studied at the same pH=8.7 with increasing nickel concentration to the final molar ratio 1:1 peptide-Ni(II). Unfortunately, in the final molar ratio of 1:1 precipitation was observed, nevertheless, clearly information on the binding mode of peptide- Ni(II) can be obtained from a series of 1D ^{1}H, 2D TOCSY and NOESY complex spectra until molar ratio 1-0.8. A minor shift of the two histidine aromatic residues (at 7.472 ppm, Δδ= 0.123 and at 6.837 ppm, Δδ= 0.025 for HE_1 and HD_2, respectively), as well as strong shifts involving the Hα and Hβ proton regions were clearly observed (Hα 3.481 ppm, Δδ= 1.032; $H\beta_1$ 2.840 ppm, Δδ= 0.202, $H\beta_2$ 2.709 ppm, Δδ= 0.261). A new spin system appears in the TOCSY and NOESY spectra for R and K residues, attributable to K_{16} and R_{17}, in which strong shifts resonance at ppm belonged to the αH protons of K (at 3.937, Δγ= 0.281 ppm) and R (at 3.565, Δγ= 0.695 ppm), were identified. The values of chemical shifts of the new system identified for K_{16} , R_{17} and H_{18} and the differences with the resonances of free peptide are reported in Table 1.

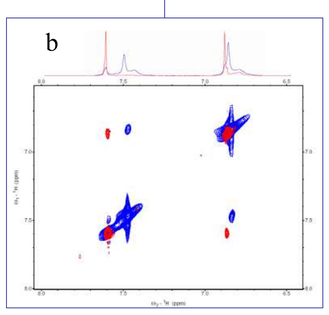


*Superimposition of Tocsy spectra of free 30aa peptide (red) and the system 30aa peptide-Ni(II) (cyan) **a)** molar ratio 1-0.2; **b)** 30aa peptide-Ni(II) (green) molar ratio 1-0.4 and **c)** 30aa peptide-Ni(II) (blu) molar ratio 1-0.8, in which is showed the new spin systems for Lys16, Arg17 and His18 after nickel interaction.*



Changes in intensity and chemical shift of the histidine aromatic proton (HE1 and HD2) by increasing nickel concentration

***a)** Comparison of aromatic region of 1D H NMR spectra of 30aa peptide Cap43-Ni(II) in the molar ratio 1-0, 1-0.4. 1-0.6, 1-0.8 and **b)** superimposition of aromatic region of 2D Tocsy of free 30aa peptide (red) and the system 30aa peptide-Ni(II) molar ratio 1-0.8*



Res-H	Pept H4 free ppm	Pept H4-Ni(II) 1-08 ppm	Δδ
K QE	2.896	2.932	-0.036
K QD	1.580	1.679	-0.099
K QG	1.355	1.499	-0.144
K HB2	1.793	2.183	-0.390
K HB1	1.687	1.969	-0.282
K HA	4.218	3.937	0.281
R QD	3.119	3.119	0.000
R QG	1.541	1.429	0.112
R HB2	1.793	2.255	-0.462
R HB1	1.683	2.019	-0.336
R HA	4.260	3.565	0.695
H HB1	3.042	2.840	0.202
H HB2	2.970	2.709	0.261
H HA	4.513	3.481	1.032
H HE1	7.595	7.472	0.123
H HD2	6.862	6.837	0.025

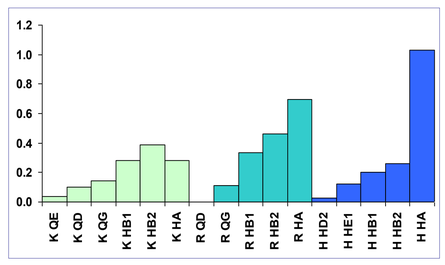
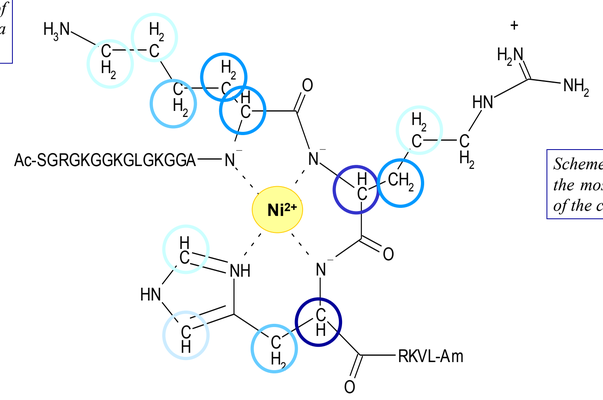


***Table 1.** Chemical shift assegnment for the residues involved in the complex formation, before and after nickel interaction. The differences of chemical shifts are reported in the plot (absolute values).*



Scheme of the Ni(II)-peptide complex. The circle indicate the most affected proton in the interaction, the hardeness of the color are proportional to the shift's changes

REFERENCE

1. E. Denkhaus ,K. Salnikow, Crit Rev Oncol Hematol. 42(1), 2002, pp. 35-56.
2. IARC, Lyon, France, *Monographs on the evaluation of carcinogenic risks to humans*. Vol. 49, 1990.
3. R.M.Evans, P.J.Davies, M.Costa, *Cancer Res.* 1982, *42*, 2729
4. L. Broday, W. Peng, M.H. Kuo, K. Salnikow, M.A. Zoroddu, M. Costa, Cancer Res. 60(2), 2000, pp. 238-4.
5. M.A. Zoroddu, M. Peana, T. Kowalik-Jankowska, H. Kozlowski, M. Costa, J. Chem. Soc., Dalton Trans., 2002, pp. 458–465
6. M.A. Zoroddu , L. Schinocca, T. Kowalik-Jankowska, H. Kozlowski, K. Salnikow , M. Costa, Environ Health Perspect., 110 Suppl 5, 2002, pp.719-23.

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