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Interaction of Ni(II) and Cu(II) with a metal binding sequence of histone H4: AKRHRK, a model of the H4 tail

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Abstract

Chromatin proteins are believed to represent reactive sites for nickel binding. The unique structure of the N-terminal tail of histone H4 contains sites for post-translational modification close to a histidine residue capable of anchoring binding sites for metal ions. We have analyzed as a minimal model for the H4 tail, the blocked peptide CH_3CO-AKRHRK-$CONH_2$ for nickel and copper binding. Ultraviolet–visible, circular dichroism, electron paramagnetic resonance and nuclear magnetic resonance spectroscopic analysis showed that histidine acts as an anchoring metal binding site. A 1N complex is formed between pH = 5–7 and 4–6 for Ni(II) and Cu(II), respectively, while at a higher pH a series of 4N complexes are formed. Above pH 8, the 2N high-spin octahedral resulted in a 4N low-spin planar Ni(II) complex. The stability constants of the Cu(II) (3N, 4N) and Ni(II) (4N) complexes with the peptide model of the H4 were distinctly higher than those for a similar blocked peptide with a histidine in the fourth position. Significant shifts in the αproton region in the ^{1}H NMR spectrum of the 4N Ni-complex showed that the conformation of the peptide had been dramatically affected following Ni(II) complexation.

Keywords: Ni(II); Cu(II); Histone H4; AKRHRK peptide

1. Introduction

Ni(II) compounds are well established human carcinogens [1,2]. Ni(II) has been shown to enhance chromatin condensation and increase DNA methylation resulting in the down regulation of gene transcription [3]. The molecular mechanism of nickel carcinogenicity, though not fully understood, is believed to involve DNA damage and epigenetic effects in chromatin resulting from nickel binding to the cell nucleus [4–6]. It is known that Ni(II) can bind DNA only weakly [7]. Therefore, the nucleus proteins are possible targets for nickel binding. The most abundant proteins of the cell nucleus are histones. Consequently, it is possible that histones may be able to compete for metal ions with even higher affinity metal binding sites in other, less abundant nuclear proteins or smaller molecules such as glutathione or histidine.

The core histones have multiple domain structures, consisting of globular carboxy-terminals and randomly coiled, very basic amino tail regions. Some of the terminal tails are involved in internucleosome contacts in the higher order structure of the crystals. One of the H4 N-terminal regions (residues 1–23) makes multiple hydrogen bonds between its basic side chain (K_{16}, R_{19}, K_{20}, R_{23}) and acidic side chains of H2A and H2B dimer of a neighboring nucleosome core [8]. DNase digestion and DNA/protein cross-linking studies have shown that N-terminal sequences of H4 are peripheral and accessible to protease degradation [9].

Histones are extensively and reversibly post-translationally modified by acetylation, phosphorylation, ubiquitination and methylation. These modifications are confined to

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the tail regions of the proteins. Of these modifications, acetylation has generated more interest since gene expression was directly correlated with histone acetylation. The site of acetylation is the lysine residues of the positively charged amino-terminal tails where each acetyl group added to a histone reduces its net positive charge weakening and modulating interaction between histones and the surface of the nucleosome. The positive charge of the H4 tail is neutralized by acetylations at lysines 5, 8, 12 and 16, increasing the access to transcription factors and making 'active chromatin' more available for modifications [10]. Studies involving the amino-terminal tail of H4 are of particular interest providing information on the regulation of chromatin structure and function. In this paper our primary interest was to investigate the putative binding sites for toxic metal ions in histones. For this purpose, we considered a binding motif in the H4 tail in that, because of its mobility, this terminal part could be accessible for metal binding. We therefore began our investigation with a minimal model of the H4 tail, the sequence AKRHRK (Ala-Lys-Arg-His-Arg-Lys, $A_{15}K_{16}R_{17}H_{18}R_{19}$-$K_{20}$), where an anchoring binding site for metal ions, a histidine, is close to sites for post-translational modification involved in nickel and also in copper toxicity.

The binding study was also extended to Cu(II) in view of the reported [11] toxicity of this metal to yeast cells. In fact, it has been reported that Cu(II) is a novel inhibitor of H4 acetylation on the lysines close to histidine 18 in the H4 tail.

To make the peptide AKRHRK a more relevant model of the H4 tail protein sequence, N- and C-termini were blocked by acetylation and amidation.

2. Material and methods

2.1. Synthesis of the peptide

The CH_3CO-Ala-Lys-Arg-His-Arg-Lys-$CONH_2$ peptide sequence was synthesized on a solid support with a 9050 Plus Synthesizer by BBM (Woburn, MA, USA) using a conventional Fmoc chemistry methodology [12] starting from the carboxy-terminal to the amino-terminal using Fmoc-L-Ala-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Arg(Pbf)-OH and Fmoc-L-His(trt)-OH (Boc = *t*-butoxycarbonyl, Pbf = pentamethyldihydro-benzofuran-5-sulfonyl, trt = trityl). *N*-Fmoc protected amino acids and all other peptide synthesis reagents were obtained from PE Biosystem, USA. The peptide was purified using semipreparative high-performance liquid chromatography (HPLC) on a C_{18} column eluting with 0.1% CF_3COOH–H_2O (solvent A) and 0.1% CF_3COOH–CH_3CN (solvent B), linear gradient 0–100% B over 50 min at a 3 ml/min flow rate with detection at 220 nm absorption used as a means of detection. The purity of the peptide was checked by reversed-phase HPLC using C_{18} 5 μm 100A microsorb-MV analytical column 4.6 mm × 25 cm, flow rate 1 ml/min, gradient 0–50% in 25 min (solvent A, 0.1% CF_3COOH–H_2O), 50–100% in 35 min (solvent B, 0.1% CH_3CN–H_2O). The molecular weight of the peptide was confirmed by mass spectral analysis (MALDI.TOF.VOYAGER).

2.2. Potentiometric measurements

Stability constants for protons, Cu(II) and Ni(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 which was calibrated by both weight titration and the titration of standard materials. The metal ion concentration was 1.5×10^{-3} mol dm^{-3} and the metal-to-ligand molar ratio was 1:1.1. The pH-metric titrations were performed at 25°C in 0.10 mol dm^{-3} KNO_3 on a MOLSPIN pH-meter system using a microcombined glass/calomel electrode calibrated in hydrogen ion concentrations using HNO_3 [13]. The SUPERQUAD computer program was used for stability constant calculations [14]. 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.

2.3. Spectroscopic measurements

Solutions were of similar concentrations to those used in the potentiometric studies. Electron paramagnetic resonance (EPR) spectra were recorded on a Bruker ESP 300E spectrometer at X-band frequency (9.3 GHz) at 120 K. The EPR parameters were calculated for the spectra obtained at the maximum concentration of the particular species for which well-resolved separations were observed. The absorption spectra were recorded on a Beckman DU 650 spectrophotometer. Circular dichroism (CD) spectra were recorded on Jasco J 600 spectropolarimeter 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. Nuclear magnetic resonance (NMR) experiments were performed on a Bruker DRX 500 spectrometer. The 1D experiments were acquired with a sweep width of 5400 Hz with 32 K points in 90/10 aqueous/D_2O solution at an Ac-AKRHRK-Am concentration of 4 mM and peptide:Ni(II) ratio of 1.1:1 at pH* 9.4; (pH* reading of the electrode not corrected for isotope effect); pH* was adjusted with NaOH 2 M and monitored on an Aldrich extra long stem electrode inside the NMR tubes. 2D TOCSY experiments [15] were carried out in the phase-sensitive mode using the TPPI method. Four hundred experiments of 2 K data points were collected. Prior to Fourier transformation, the time domain data was multiplied by a Gaussian window function in both dimensions. The water peak was suppressed with the Watergate sequence.

Table 1
Stability constants of complexes of H^+, Cu^{2+} and Ni^{2+} with Ac-AKRHRK-Am and comparable ligand at 25°C and $I = 0.10$ mol dm^{-3} (KNO_3)

Protonation constants (log values)						
	β(HL)	β(H_2L)	β(H_3L)	K_{imid}	K_{NH_2Lys}	K_{NH_2Lys}
Ac-AKRHRK-Am	11.03 ± 0.01	20.90 ± 0.01	27.03 ± 0.01	6.13	9.87	11.03
Boc-AGGH[a]	7.19	10.02		7.19		
Copper complexes stability constants (log β values)						
	CuH_2L	CuL	$CuH_{-1}L$		$CuH_{-2}L$	$CuH_{-3}L$
Ac-AKRHRK-Am	23.70 ± 0.03	12.45 ± 0.01	3.98 ± 0.01		−5.94 ± 0.01	−16.92 ± 0.02
Boc-AGGH[a]		4.97	−1.82		−8.49	−17.42
Log K*	1N	2N	3N		4N	
Ac-AKRHRK-Am	−3.33		−14.58		−23.05	
Boc-AGGH[a]	−2.22	−9.01	−15.68		−24.61	
Nickel complexes stability constants (log β values)						
	NiH_2L	NiHL	$NiH_{-1}L$		$NiH_{-2}L$	$NiH_{-3}L$
Ac-AKRHRK-Am	23.01 ± 0.06	15.05 ± 0.04	−1.67 ± 0.01		−11.80 ± 0.02	−22.84 ± 0.03
Boc-AGGH[b]			−4.49			−22.83
Log K*	1N	2N	4N			
Ac-AKRHRK-Am	−4.02	−11.98	−28.70			
Boc-AGGH[b]		−11.68	−30.02			

Log $K^* = \log\beta(MeH_{-n}L) - \log\beta(H_3L)$ (H_3L with Ac-AKRHRK-Am and HL with Boc-AGGH.
[a][16]
[b][17]

3. Results

3.1. Protonation constants

The potentiometrically measured protonation constants are shown in Table 1 along with the calculated stepwise constants assigned to the respective peptidic functions. For comparison, the calculated constants were reported for Ni(II) and Cu(II) binding to a peptide like Boc-AGGH [16–17]. This peptide is comparable to our model because it has a histidine in the fourth position following an alanine N-terminal blocked as in our sequence. From this comparison we see that the stability constants are either of the same order of magnitude or higher for our sequence CH_3CO-AKRH... than for Boc-AGGH.

As we can see from the values reported in Table 1, the pK values of the two lysine side chain amino groups differ by distinctly more than 0.6 log units, therefore, they could be candidates for interaction. The imidazole nitrogen of the histidine residue is one order of magnitude more acidic than that of the Boc-AGGH peptide.

3.2. Cu(II) complexes

The stability constants and stoichiometry of the complexes are given in Table 1. Four species, CuL, $CuH_{-1}L$, $CuH_{-2}L$ and $CuH_{-3}L$, were the major complexes and their spectroscopic parameters were assigned. The former complex CuL, with a d–d transition at 593 nm (absorption spectra) and EPR parameters shown in Table 2, is the 3N complex with three nitrogens bound to a metal ion [16,18–19]. It is interesting to note that the stability of this complex is considerably higher than the analogous species Boc-AGGH in the same coordination mode. The log K* value, which is characteristic for the removal of two amide protons, is more than one order of magnitude higher for the title peptide than that for the Boc-AGGH peptide (Table 1). Three other species formed by the Ac-AKRHRK-Am peptide, i.e. $CuH_{-1}L$, $CuH_{-2}L$ and $CuH_{-3}L$, are the 4N complexes with various protonation states of the side chain lysine residues (two, one or none). The CD spectra clearly indicate the binding of imidazole and amide nitrogens to Cu(II) ion (Table 2) [16,18–20]. The imidazole nitrogen acts as the metal ion (the anchoring group), while three amide nitrogens, i.e. those of His, Arg and Lys residues, complete the co-ordination in a stepwise mode [16,18–19]. It is interesting to note that

Table 2
Spectroscopic data for the Cu(II) and Ni(II) complexes of Ac-AKRHRK-Am

Species	Absorption	CD	EPR	
	λ (nm) (ε)[d]	λ nm (Δε)	$g_{\parallel}$	$A_{\parallel}(G)$
Cu^{2+} complexes				
CuL (3N)	593[a] (84)	535 (+0.687)[a]	2.224	174
		337 (−0.806)[b]		
$CuH_{-1}L$ (4N)	513[a] (108)	626 (+0.659)[a]	2.185	201
		482 (−0.897)[a]		
		350 (−0.398)[b]		
		310 (+0.667)[c]		
$CuH_{-2}L$ (4N)	516[a] (114)	625 (+0.961)[a]	2.186	204
		484 (−1.322)[a]		
		353 (−0.281)[b]		
		313 (+1.037)[c]		
Ni^{2+} complexes				
NiH_{-1} L (4N)	438 (162)	507 (+0.888)[a]		
	487sh (116)	416 (−3.993)[a]		
$NiH_{-2}L$ (4N)	439 (166)	506 (+0.785)[a]		
	487sh (121)	413 (−4.511)[a]		

[a]d–d transition.
[b]$N_{imidazole} \Rightarrow Cu^{2+}$ charge transfer transition.
[c]$N^- \Rightarrow Cu^{2+}$ charge transfer transition.
[d]From the visible and CD results, dm^3 mol^{-1} cm^{-1}.

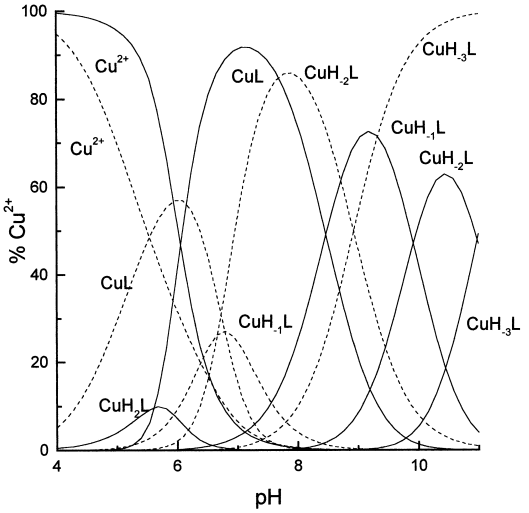

Fig. 1. Comparison of the distribution diagram of the species for a 1:1 mixture of Cu(II) (1.0×10^{-3} mol dm^{-3}) with Ac-AKRHRK-Am (black line), and of Cu(II)-Boc-AGGH species (dashed line) [16].

the deprotonation of the two first amide nitrogens occurs almost simultaneously and the 2N species was not detected (Table 1 and Fig. 1). The stabilities of the 3N and 4N complexes formed by the H4 hexapeptide are distinctly higher (expressed, for example, by $\log K^*$, Table 1) than for simple Boc-AGGH peptide. It is clearly seen in the species distribution diagram (Fig. 1) that 3N and 4N complexes are formed at a pH about one unit lower for the H4 peptide when compared to the Boc-AGGH peptide.

3.3. Ni(II) complexes

A histidine residue can be a basic binding site for Ni(II) ions in the histone core of the nucleosome. Therefore, the H4 tail peptide fragment can potentially be one of the biologically relevant sites for nickel genotoxicity. The coordination ability of the hexapeptide towards Ni(II) is similar to that described above for Cu(II). Ni(II) forms five complex species, from the minor octahedral NiH_2L 1N complex up to the three square planar 4N species: $NiH_{-1}L$, $NiH_{-2}L$ and $NiH_{-3}L$. The absorption and CD spectra of the latter complexes are typical for 4N planar complexes [17,21–22]. The 4N species are the only major complexes seen in the solutions studied indicating a very effective cooperative effect usually observed for Ni(II)–peptide complexes [18,23]. As in the case of Cu(II) complexes, the stability constants of the 4N species of the H4 peptide are distinctly higher than those for the Boc-AGGH peptide (Table 1 and Fig. 2).

A comparison of the 1D NMR spectra of Ac-$A_1K_2R_3H_4R_5K_6$-Am and of Ac-$A_1K_2R_3H_4R_5K_6$-Am-Ni(II) species (Fig. 3) was performed at pH* = 9.4. 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 six residues of the free peptide were assigned on the basis of 1D NMR spectra and 2D 1H homonuclear TOCSY experiments. In the region between 6.6 and 8.5 ppm, only the aromatic resonances of histidine H_2 and H_4 at 7.58 and 6.86 ppm, respectively, were present. All the amide resonances were in a fast exchange with water at this pH and their resonances were lost. One residual weak signal at 8.36 ppm can be tentatively assigned to the ε NH of the two arginines. In the aliphatic region, only the α proton of histidine was not overlapped and appeared at 4.5 ppm. Its assignment was based on the analysis of the TOCSY spectrum, where a correlation between α H histidine and its β protons at 2.95 ppm was visible. The remaining α protons were broadly overlapping at 4.1–4.2 ppm. Integration of the two regions at 4.5 and 4.2 ppm afforded a 1:5 ratio, as expected. The multiplets at 3.1 and 2.75 ppm were assigned to δ H of R_3, R_5 and to ε H of K_2 and K_6, respectively. The TOCSY spectrum allowed the assignment of the entire spin system of the arginine and lysine residues. The R γ protons resonated at 1.5 ppm, and the β protons at 1.62 and 1.72 ppm. The assignment for the lysine spin system was ε H at 2.75 ppm, δ H at 1.5 ppm, γ H at 1.3 ppm and β H at 1.68 and at 1.72 ppm. The spin system of alanine was easily assigned as it showed a doublet at 1.25 ppm, exhibiting a unique correlation in the TOCSY spectrum with the multiplet centered at 4.18 ppm. This integration agrees with the previous assignment. After addition of Ni(II) at pH* = 9.4, we obtained a complex spectrum not easily amenable to interpretation. It is clear that some free peptide was still present in solution. The ratio of free to bound peptide appeared to be 1:4 on the basis of integrals. The broadening of the signals was probably due to the slow kinetics of the $NiH_{-1}L$ species. Nevertheless, some information on the binding mode of Ni(II) to the H4 sequence can be obtained. A minor shift of the two histidine aromatic residues (at 7.513 ppm, $\Delta\delta = -0.067$ and at 6.834 ppm,

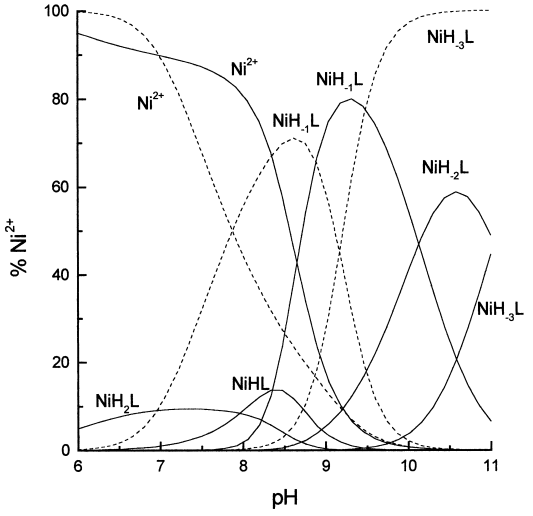

Fig. 2. Comparison of the distribution diagram of the species for a 1:1 mixture of Ni(II) (1.0×10^{-3} mol dm^{-3}) with Ac-AKRHRK-Am (black line) and of Ni(II)-Boc-AGGH species (dashed line) [17].

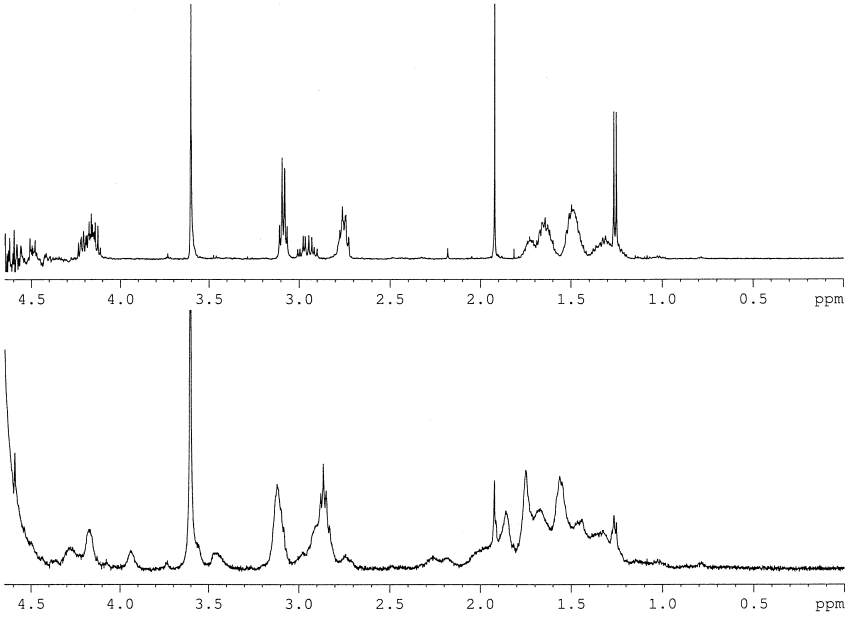

Fig. 3. The 500 MHz 1H NMR spectra of (a) Ac-AKRHRK-Am and (b) Ac-AKRHRK-Am-Ni(II) complex at pH* = 9.4.

$\Delta\delta = -0.026$ for H_2 and H_4, respectively), as well as shifts involving the α proton regions were clearly observed. The most intense resonance at 4.18 ppm belonged to the α protons of K and R, as confirmed in the TOCSY spectra. The broad signal at ca. 4.28 ppm which can be attributed to the α alanine protons, appeared to correlate with the resonance at 1.93 ppm, suggesting the presence of an alanine spin system, shifted downfield ($\Delta\delta$ = ca. +0.11 ppm) upon complexation. A strong shift for the α and β protons of histidine was detected: the α histidine protons were located at ca. 4.1 ppm, with a $\Delta\delta = +0.4$, and β protons at ca. 2.75 ppm, with a $\Delta\delta = +0.2$. Also, a new spin system appears in the TOCSY spectra for R and K residues but they could not be assigned unambiguously from our data.

4. Discussion

The present paper reports the results of a study on the interaction of Ni(II) and Cu(II) with a minimal model of the H4 tail.

The characterization of metal ion interactions with an entire histone is a difficult task. Therefore, we began our investigation with a minimal model with the aim of reproducing local binding capabilities of the corresponding sequence of amino acids in the H4. The next step of our studies will be to find whether our motif is a nickel and copper binding site in the histone H4. Although we don't obtain much complexation for Ni(II) at a physiological pH under our conditions, the hydrophobic environment in the entire protein is expected to enhance metal binding capabilities, due to multiple non-bonding interactions available there, as reported in the literature [24–26].

The results presented above show that Ac-AKRHRK-Am binds Ni(II) and Cu(II) through the imidazole nitrogen at pH 5 and at pH 4, respectively, giving 1N complexes; when the pH was increased both metal ions deprotonated successive peptide nitrogens, forming $M\text{-}N^-$ bonds, until a $MH_{-3}L$ species (4N complexes) was formed above pH 8. The formation of stable five-membered chelate rings by consecutive nitrogens is the driving force of the coordination process. Although the imidazole nitrogen of the histidine in the H4 model was one order of magnitude more acidic than that of the Boc-AGGH peptide, reported for comparison, the stability constants of the 3N, 4N complexes of Cu(II) and the 4N complex of Ni(II) with the peptide model of the H4 were considerably higher than the analogous species of Boc-AGGH in the same coordination mode. This fact suggested that the presence of positively charged side chains of lysines and arginines may have increased the stability of the 3N, 4N complexes [27].

The proton NMR spectra showed significant downfield and upfield changes particularly with regard to the αCH protons of the backbone as a result of complexation of Ni(II) to the H4 model Ac-AKRHRK-Am. The strong downfield shift of the αCH alanine supports the involvement of the alanine terminal part of the H4 sequence, suggesting that structural changes were involved at the N-terminal tail of the H4 sequence following metal complexation. Additionally, while the δ arginine protons showed little effect, ε lysine protons exhibited a downfield shift, suggesting a change of hydrophobic packing of the lysine side chains following nickel binding. When Ni(II) and Cu(II) form 4N complexes with a peptide ligand, the conformation of the peptide will be dramatically affected; this aspect is particularly important with donors able to bind metal ions through the backbone peptide nitrogens

since they will influence both the physical and biological properties of the peptide.

In conclusion, our results show that Ac-AKRHRK-Am, a minimal model of the H4 tail, could be a competitive binding site for nickel as well as for copper.

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