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Research Article

Cu(II) and Ni(II) Interactions with the Terminally Blocked Hexapeptide Ac-Leu-Ala-His-Tyr-Asn-Lys-amide Model of Histone H2B (80–85)

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The N- and C-terminal blocked hexapeptide Ac-Leu-Ala-His-Tyr-Asn-Lys-amide (LAHYNK) representing the 80–85 fragment of histone H2B was synthesized and its interactions with Cu(II) and Ni(II) ions were studied by potentiometric, UV-Vis, CD, EPR, and NMR spectroscopic techniques in solution. Our data reveal that the imidazole N(3) nitrogen atom is the primary ligating group for both metal ions. Sequential amide groups deprotonation and subsequent coordination to metal ions indicated an $\{N_{imidazole}, 3N_{amide}\}$ coordination mode above pH∼9, in all cases. In the case of Cu(II)-peptide system, the almost exclusive formation of the predominant species CuL in neutral media accounting for almost 98% of the total metal ion concentration at pH 7.3 strongly indicates that at physiological pH values the sequence -LAHYNK- of histone H2B provides very efficient binding sites for metal ions. The imidazole pyrrole N(1) ionization (but not coordination) was also detected in species $CuH_{-4}L$ present in solution above pH ∼ 11.

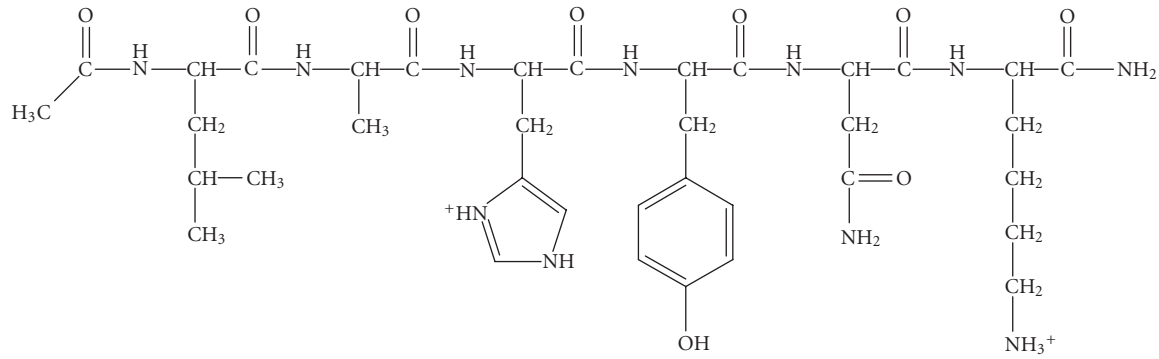
1. INTRODUCTION

In eukaryotes, the genome is organized as a highly complex and condensed structure called chromatin, the unit of which is the nucleosome. It is well known that about 146-147 DNA base pairs are bound around a histone octamer which contains two copies of each of histones H2A, H2B, H3, and H4, forming the nucleosomes [1]. Chemical and structural description of the possible metal binding sites inside the cell nuclei could provide the molecular basis for a better understanding of the interactions responsible for cancer or similar genetic disease developments. It is believed that the binding abilities of the metal ions inside the cell nuclei may have repercussions in the genetic code. Having in mind the efficiency of metal ion coordination to proteins and DNA and the plenitude of histones into the nucleosome core, it is assumed that histones can be the fundamental binding sites for metal ions.

In this respect, previous studies have shown that certain histone peptide model, fragments of H2A, H2B, H3, and H4 may serve as efficient binding sites for metal ions such as Cu(II) and Ni(II) [2–14]. Moreover, interactions of metal ions with small peptides have revealed that His residues are the major coordination sites of several transition metal ions, including Ni(II) and Cu(II), in proteins [15–18].

Our research group initial studies in this interesting area, included the coordination abilities of Ni(II) and Cu(II) ions towards a series of terminally blocked hexapeptide models of the C-terminal "tail" -ESHH- of histone H2A. It was found that Ni(II) ions promoted hydrolysis of the peptides -TASHHK- and -TESAHK- at the -X-Ser- sites and this was assigned to the presence of the Ser residue [6–8, 10, 13] with an –OH group near the coordination site.

The driving force of the hydrolysis reaction of H2A histone blocked hexapeptide models was the high thermodynamic stability of Ni(II) or Cu(II) complexes with the



Scheme 1: The hexapeptide Ac-Leu-Ala-His-Tyr-Asn-Lys-amide (-LAHYNK-).

hydrolysis products -SHHK- and -SAHK-, respectively [10]. Continuing our work, we have recently reported the efficient binding of the blocked hexapeptide -ELAKHA- model of the C-terminal of histone H2B to Cu(II) and Ni(II) ions [11], and in a more comprehensive study [14] the binding abilities of the C-terminal 71–76 fragment of histone H4 Ac-ThrTyrThrGluHisAla-amide in which Ni(II) mediated hydrolysis of the peptide bond Tyr-Thr was evident [14], in agreement with previous findings concerning the hydrolytic cleavage of the peptide bond Glu-Thr of the peptide -TETHHK- [9].

Taking into account, the variety of possible coordination sites of histones plus the great anchoring capability of the histidyl residues towards Cu(II) and Ni(II) ions as stated above, we decided to continue our investigation with the new hexapeptide model -LAHYNK-, the 80–85 fragment of histone H2B (Scheme 1) which, according to the X-ray crystal structure of the nucleosome core [1], may be accessible for metal ions binding.

To make the peptide a more reliable model of the H2B 80–85 protein sequence, N- and C-termini were blocked by acetylation and amidation, respectively. A combination of potentiometric and spectroscopic (UV-Vis, CD, EPR, NMR) studies were applied in order to evaluate the formation models, species stoichiometry and possible coordination modes reported in this work.

2. EXPERIMENTAL

2.1. Materials

Trifluoroethanol (TFE), 1-hydroxybenzotriazole (1-HOBt), anisole, trifluoroacetic acid (TFA), 3-(trimethylsilyl)propionic acid sodium salt (TSP), D_2O, and DCl were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, Wis, USA). $Cu(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, HNO_3, KNO_3, acetonitrile (HPLC grade), dicyclohexylcarbodiimide (DCC), ethanediol, HCl, and KOH were obtained from E. Merck (Darmstadt, Germany). Diethyl ether, isopropanol, dimethylformamide, and dichloromethane were purchased (analytical grade) from Labscan Chemical Co. (Dublin, Ireland). The resin H-Linker-2-chlorotrityl and the protected amino acids, Fmoc-His(*Mtt*)-OH, Fmoc-Lys(*Boc*)-OH, Fmoc-Leu-OH, Fmoc-Asn-OH, Fmoc-Tyr(OBu^t), and Fmoc-Ala-OH, were purchased from CBL Chemicals Ltd. (Patras, Greece).

2.2. Methods

2.2.1. Peptide synthesis

The blocked hexapeptide Ac-Leu-Ala-His-Tyr-Asn-Lys-amide (-LAHYNK-) (Scheme 1) was synthesized in the solid phase using the Fmoc chemistry [19, 20]. 1-hydroxybenzotriazole (1-HOBt) and dicyclohexylcarbodiimide (DCC) were used as coupling reagents, where Fmoc protection groups were removed before each coupling step by a 25% (v/v) piperidine solution in DMF. The removal of the protecting groups *Mtt*-, *Boc*-, and *Bu^t*- was accomplished by a 65% TFA in CH_2Cl_2:TFE (6:1 v/v) solution and 5% anisole, as described in the literature [19, 20]. The peptide cleavage from the resin was performed using a CH_2Cl_2:TFE:CH_3COOH (7:2:1 v/v) solution. The crude peptide was purified by preparative HPLC (Dionex system equipped with a P 580 pump) on a Waters C_{18} column (300 × 9 mm, 15 μm, 300 Å) and its purity was finally confirmed by means of one-/two-dimensional ^{1}H-NMR techniques (Varian Unity 500 MHz).

2.2.2. Potentiometry

Potentiometric titrations of free hexapeptide and its complexes with Cu(II) and Ni(II) ions were carried out at 25°C, using a total volume of 2.0 mL. The titrations were performed in the presence of 0.1 M KNO_3 over the pH range 2.5–11 on a MOLSPIN pH-meter system (Molspin automatic titrator, Molspin Ltd., Newcastle-upon-Tyne, U.K.), using a 0.500 mL micrometer syringe and a combined glass-silver chloride electrode calibrated in hydrogen concentrations using HNO_3 [21]. 0.1 M KOH was used as titrant. The concentrations of the hexapeptide and the metal ions were between 2 mM and 1 mM at metal:peptide molar ratio between 1 : 1 and 1 : 2. For the binary system -LAHYNK-/Ni(II), excess of the hexapeptide (1 : 2) had been used to achieve shorter equilibrium times, due to the slow kinetics of the formation of the square-planar nickel complexes at a

Table 1: Chemical shifts of ^{1}H (δ, ppm) of free and Ni(II)-bound -TYTEHA-, in a metal:peptide ratio 1 : 1, at pH* 9.5.

-LAHYNK-	H_α	H_β	H_γ	Others
Ac				1.95
^{1}Leu	4.22 <u>4.11</u> (−0.11)*	4.03	1.44	δ-$CH_3\alpha$:0.81, δ-$CH_3\beta$:0.85
^{2}Ala	4.19 <u>3.38</u> (−0.81)*			CH_3:1.20
^{3}His	4.46 <u>3.86</u> (−0.60)*	2.86, 2.92		H_2 7.61 <u>7.25</u> (−0.36)* H_5 6.82 <u>6.77</u> (−0.05)*
^{4}Tyr	4.44 <u>4.64</u> (+0.20)*	2.86, 2.92	2.14	$H_{2,6}$ 6.97 $H_{3,5}$ 6.70
^{5}Asn	4.60 <u>4.60</u> (0.00)*	2.61, 2.73		
^{6}Lys	4.16 <u>4.23</u> (+0.07)*	1.67, 1.79	1.36	H_δ:1.61, H_ε:2.92

* Underlined the proton chemical shifts of the bound imidazole ring and C_a-H (the chemical shift difference in parenthesis).

Table 2: Stability and ionization constants of the peptide -LAHYNK-.

	Overall protonation constants ($\log\beta$)[(a)]					
-LAHYNK-	HL	H_2L	H_3L	pK_{His}	pK_{Tyr}	pK_{Tys}
	10.53 (2)	20.05 (2)	26.28 (3)	6.23	9.52	10.53

[(a)] $\beta = [H_jL_k]/([H]^j\,[L]^k)$, standard deviations of the last digit are given in parenthesis.

ratio 1 : 1. The experimental data were analyzed using the HYPERQUAD program [22]. Standard deviations computed by HYPERQUAD refer to random errors.

2.2.3. NMR spectroscopy

NMR experiments were performed on a Varian Unity spectrometer at 500 MHz. The one-dimensional ^{1}H-NMR experiments were performed in 99.9% D_2O solutions at a peptide concentration of 7 mM, (pH* 9.5 at 25°C) in the absence or presence of Ni(II) ions (metal:peptide molar ratio 1 : 2). It must be mentioned that the pH* reading of the electrode was not corrected for the isotope effect. Additionally, ^{1}H-^{1}H-TOCSY experiments were used to assign the one-dimensional spectra of both free- and Ni(II)-bound peptide.

2.2.4. CD spectroscopy

CD spectra were recorded on a Jasco J-810 spectropolarimeter, in the spectral range of 190–800 nm and pH range 3–12, in 0.1 cm cuvettes, using 2 mM of Cu(II) and 2.5 mM of Ni(II) ions. The used Cu(II):peptide and Ni(II):peptide molar ratios were 1 : 1.1 and 1 : 2, respectively. The temperature was controlled by a Peltier heating/cooling system from 5°−90°C (278–363 K) (±0.1°C). Ten-twenty accumulations were averaged using a 5 nm band width, a 500 nm/min scanning speed, and a 0.1 or 0.5 nm data pitch. Additionally, a background subtraction was carried out for all the spectra using similar parameters.

2.2.5. UV/Vis spectroscopy

The absorption spectra of the complexes were recorded on a Jenway 6400 spectrophotometer in the spectral range of 320–900 nm, using 1 cm cuvettes. The spectra were recorded over pH range 3.5–11 by adding small amounts of concentrated KOH solution manually. Changes of the pH were monitored by a combined glass-silver chloride electrode. The used Cu(II):peptide and Ni(II):peptide ratios were 1 : 1.1 and 1 : 2, respectively.

2.2.6. EPR spectroscopy

The X-band EPR spectra were obtained at 120 K on a Varian E109 spectrometer, using 4 mM of hexapeptide at Cu(II):peptide molar ratio 1 : 1.1. Ethanediol aqueous solution (20% v/v) was used as a solvent to ensure homogeneity of the frozen samples.

3. RESULTS AND DISCUSSION

3.1. Peptide characterization and protonation constants

Our peptide -LAHYNK- (Scheme 1) was acetylated and amidated in the N- and C-terminal, respectively in order to simulate a more realistic model of the histone H2B 80–85 sequence and it was characterized by means of NMR spectroscopy. NMR chemical shift values are presented in Table 1.

Overall protonation and ionization constants (pK_a) derived by the potentiometric measurements on the free ligand are collected in Table 2.

The peptide can be considered as a triprotic acid (H_3L) due to the presence of three ionizable groups in the measurable pH range (2–12), which are, the imidazole N(3) atom of histidine, the phenolic hydroxyl group, and the ε-amino group of the Lys side chain. The first pK_a value 6.23 is assigned to imidazole N(3)-H ionization while the next two (9.52, 10.53) to Tyr-phenolate, and lys-ε-NH_2 groups, respectively. The above data are in good agreement with literature values corresponding to the above ionization types [7, 23, 24].

Table 3: Stability constants of Cu(II) and Ni(II) complexes of -LAHYNK- at 25°C.

Stability constants of Cu(II) complexes ($\log\beta$)[a]						
	CuH_2L	CuL	$CuH_{-1}L$	$CuH_{-2}L$	$CuH_{-3}L$	$CuH_{-4}L$
$pK(n/n-1)$[b]	23.55 (2)	12.06 (2)	3.02 (3)	−6.89 (2)	−17.42 (3)	−29.07 (4)
	2/0	0/−1	−1/−2	−2/−3	−3/−4	
	5.75[c]	9.04	9.91	10.53	11.65	
Stability constants of Ni(II) complexes ($\log\beta$)[a]						
	NiH_2L	NiHL	$NiH_{-1}L$	$NiH_{-2}L$	$NiH_{-3}L$	
$pK(n/n-1)$[b]	23.26 (2)	14.65 (4)	−2.60 (2)	−12.35 (2)	−23.09 (3)	
	2/1	1/−1	−1/−2	−2/−3		
	8.61	8.63[c]	9.75	10.74		

[a] $\beta = [M_iH_jL_k]/([M]^i[H]^j[L]^k)$ where M = Cu(II) or Ni(II), standard deviations of the last digit are given in parenthesis. [b]For the ionization reaction $MH_nL \leftrightarrow MH_{n-1}L + H^+$, [c]mean p$K$ value for two protons release.

Table 4: Spectroscopic parameters of Cu(II) and Ni(II) complexes of -LAHYNK-.

Species	UV/Vis	CD	EPR	
	λ_{max} (ε/M^{-1} cm^{-1})	λ_{max} ($\Delta\varepsilon$/M^{-1} cm^{-1})	$A_{\|\|}$(G)	$g_{\|\|}$
Cu(II) complexes				
CuH_2L (1N)	*	331 (−0.33)[b] 691 (0.11)[a]	*	
CuL (3N)	591 (110)	338 (−0.98)[b, c] 637 (+0.30)[a]	170	2.230
$CuH_{-1}L$ (3N)	582 (94)	299 (+0.86)[c] 339 (−0.68)[b]	173	2.231
		637 (+0.26)[a]		
$CuH_{-2}L$ (4N)	528 (120)	297 (1.00)[c], 343 (−0.39)[b]	196	2.190
		475 (−0.54)[a] 622 (+0.39)[a]		
$CuH_{-3}L$ (4N)	518 (140)	300 (+1.05)[c], 348 (−0.15)[b]	196	2.180
		484 (−0.93)[a] 614 (0.55)[a]		
$CuH_{-4}L$ (4N)	515 (150)	302 (+0.58)[c] 346 (−0.17)[b]	196	2.180
		490 (−1.30)[a] 624 (0.66)[a]		
Ni(II) complexes				
NiH_2L (1N)	*	*	—	
NiHL (2N)	*	329 (+0.34)[b, c]	—	
		416 (−0.94)[a]		
$NiH_{-1}L$ (4N)	422 (84)	336 (+0.06)[b, c] 564 (+1.08)[a]	—	
		416 (−1.23)[a]		
$NiH_{-2}L$ (4N)	434 (140)	338 (−0.03)[b, c] 514 (+1.36)[a]	—	
		416 (−2.23)[a]		
$NiH_{-3}L$ (4N)	436 (130)	336 (+0.32)[b, c] 514 (+0.80)[a]	—	
		416 (−3.32)[a]		

[a]d-d transitions of metals, [b]CT $N_{im} \rightarrow M$, [c]$N^- \rightarrow M$, where M = Cu(II) or Ni(II).
*These species could not be detected spectroscopically because of their low concentrations and/or overlap with others.

3.2. Cu(II) complexes

The interaction of Cu(II) and the hexapeptide was studied in aqueous solutions in 1 : 1 and 1 : 2 molar ratios. The titration curves were best fitted assuming the formation of six species over the pH range 3–12, namely, CuH_2L, CuL, $CuH_{-1}L$, $CuH_{-2}L$, $CuH_{-3}L$, and $CuH_{-4}L$. The stability constants of the above Cu(II) complexes with the hexapeptide -LAHYNK- are presented in Table 3 and the species distribution diagram of Cu(II) complexes is presented in Figure 1.

For the assignment of the species UV/Vis, CD, and EPR spectra were also recorded over the pH range 3–12 and in a Cu(II): peptide molar ratio 1 : 1 (Figure 2). The spectroscopic parameters are depicted in Table 4.

At acidic pH, CuH_2L species predominate. Full spectroscopic characterization was not possible due to the low concentration of the complex and the overlapping with free Cu(II) and the CuL species distribution curves (Figure 1). Nevertheless, the log K value for the reaction Cu(II) + $H_2L \leftrightarrow CuH_2L$ $\{\log K = \log\beta(CuH_2L) - \log\beta(H_2L)\}$ in our system (3.5) is comparable with literature data for the

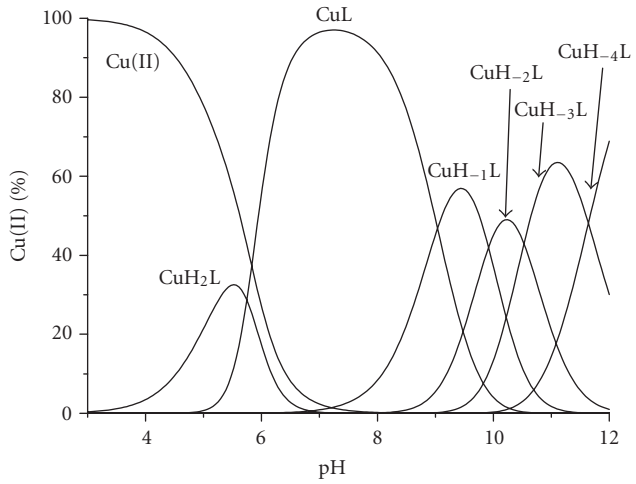


Figure 1: Species distribution diagram of Cu(II) complexes with -LAHYNK- (1 : 1), $C_{peptide}$ = 2 mM.

same type of reactions describing the formation of Cu(II)-imidazole bound complexes in the protected peptides -FKHV- (3.60), -MKHV- (3.70), and -IKQHT- (3.74) [25, 26]. Higher values were observed in the case of peptides -ELAKHA- (4.29), [11] -TESAHK- (4.47), and -TESHAK- (4.51) [7]. The difference is attributed to the stabilizing effect of the γ-carboxylate Glu residue in -ELAKHA-, -TESAHK-, and -TESHAK-. Such an effect is missing in our system.

The following species CuL is detected in neutral pH (Figure 1). Its very high concentration at pH ~ 7.3 which accounts for almost 98% of the total Cu(II) ion present and the wide pH range of existence (pH ~ 5–10.5) provide an indirect proof of its enhanced thermodynamic stability. CuL stoichiometry implies that a two proton ionization process took place cooperatively from the former species CuH_2L ($1N_{im}$ coordination mode) to CuL (3N coordination mode). Similar cooperativity has already been published for other peptide complexes containing histidyl residues [15, 17, 27]. The mean pK_a value for the ionization reaction $CuH_2L \leftrightarrow CuL + 2H^+$ is 5.75 (Table 3) and it is comparable to pK_a values for peptides with two amide nitrogens coordination (pK_a ~ 5.81–6.08) [23]. Additionally, the spectroscopic parameters of this species presented in Table 4 suggests a $3N\{1N_{im}, 2N_{amide}\}$ coordination mode according to literature data [7, 28–30]. The possible structure of this complex is depicted in Scheme 2 and it involves the equatorial coordination of the peptide through the imidazole and the amide nitrogens from His and Ala residues forming two stable, six- and five-membered chelate rings. The high-thermodynamic stability of complex CuL accounts for both the amide coordination cooperativity observed in this system and the suppression of the subsequent amide group binding.

In more basic solutions, a third proton was titrated (pK_a = 9.04) and species with stoichiometry $CuH_{-1}L$ were formed. The identical spectroscopic parameters of this species compared to CuL (Table 4) indicate that the source of this extra proton is not amide nitrogen. On the other

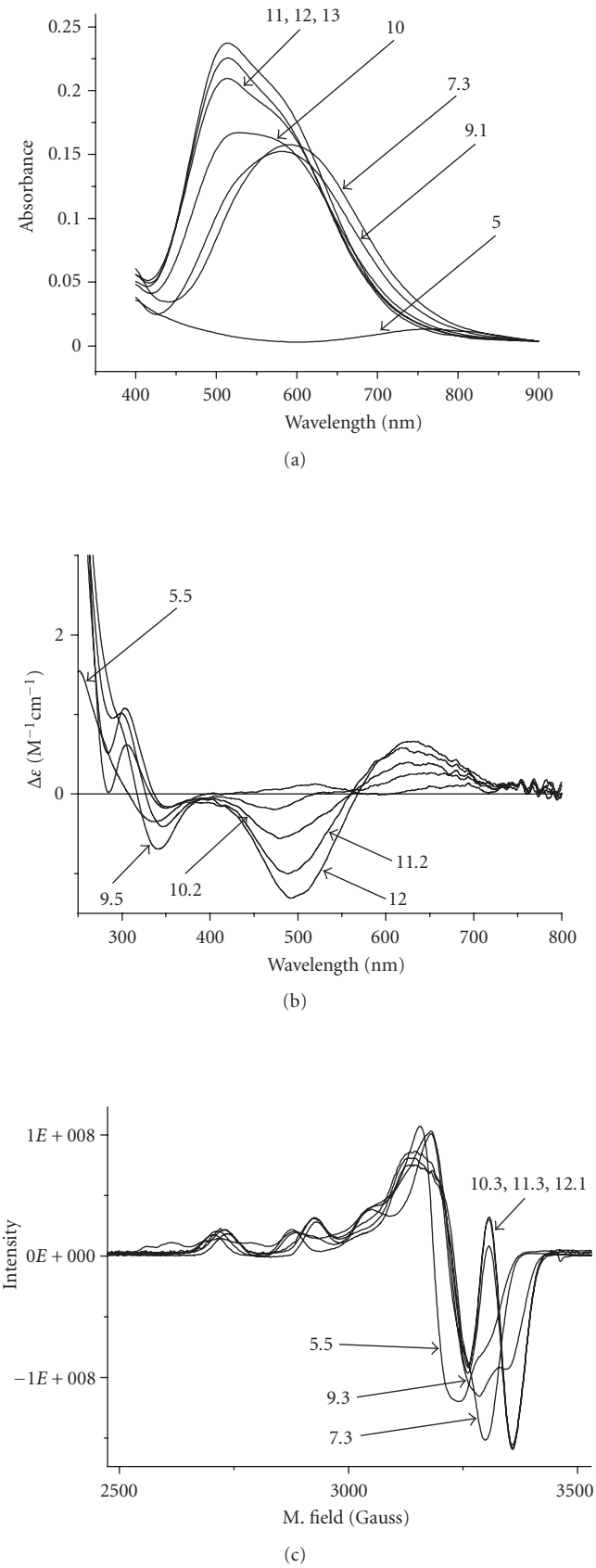
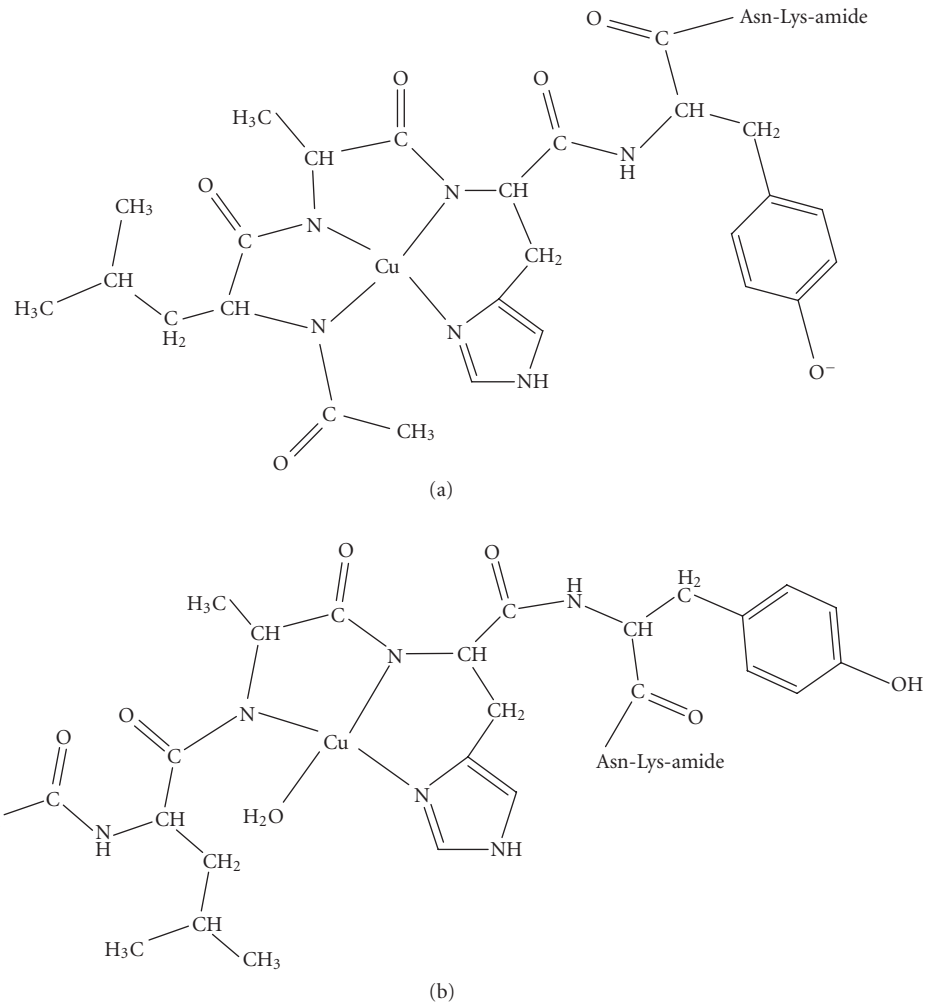


Figure 2: (a) UV/Vis, (b) CD and (c) EPR spectra of the system Cu(II)/-LAHYNK- (1 : 1) at various pH values.



Scheme 2: Possible structures of (a) $CuH_{-2}L$ (b) CuL.

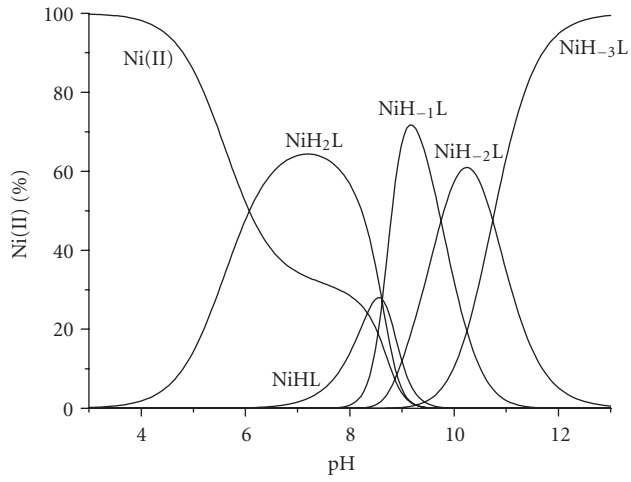


Figure 3: Species distribution diagram of Ni(II) complexes with -LAHYNK- (1 : 2), $C_{peptide}$ = 2 mM.

hand, the pK_a value already mentioned is close to that of the phenolic –OH group of Tyr residue in the free ligand (pK_a = 9.52). This suggests the same binding modes of the studied hexapeptide in both CuL and $CuH_{-1}L$ complexes, differing only in the deprotonated but uncoordinated phenolic –OH group, in the case of the latter complex.

In contrast, the UV-Vis and EPR spectroscopic parameters of the next forming species $CuH_{-2}L$ are totally different (Table 4). A blue shift of the d-d band of about 54 nm (UV-Vis) the decrease of the $g_{||}$ and concomitant increase of $A_{||}$ (EPR) suggests a stronger ligand field around Cu(II) ion due to coordination of an additional nitrogen donor. Our spectroscopic data in Table 4 suggesting a 4N donor set are in excellent agreement with data concerning species presenting the same coordination mode in histidine-containing peptides Ac-FKHV-NH_2 (λ_{max} = 528 nm, $g_{||}$ = 2.185, $A_{||}$ = 195 G), [25] Ac-GGHG-OH, ($g_{||}$ = 2.20, $A_{||}$ = 192 G) [31], and -TESAHK- (λ_{max} = 558 nm, $g_{||}$ = 2.18, $A_{||}$ = 198 G) [7]. The observed high pK_a value for the third amide ionization reaction (9.91) may be explained if we take into account that (a) deprotonation of an acetamido-group is less facilitated than that of the peptide bond [23] (b) the high stability of species CuL, (c) the negatively charged phenolic group of Tyr in species $CuH_{-1}L$.

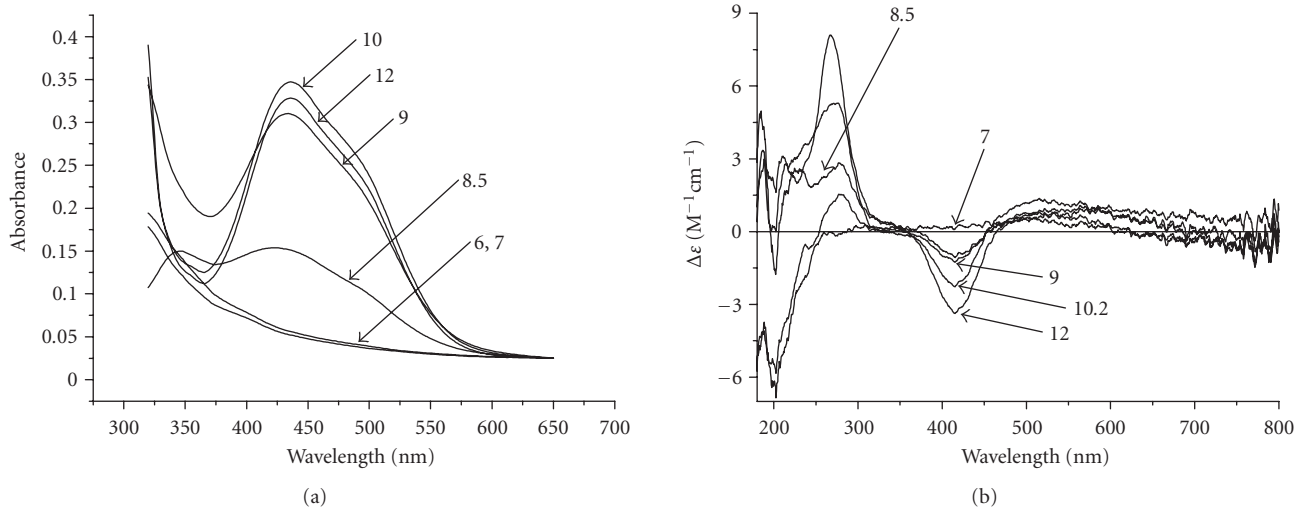
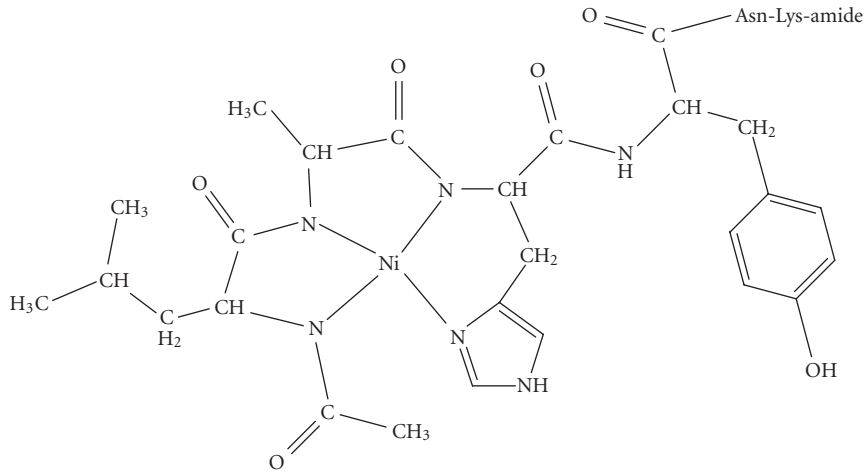


Figure 4: (a) UV/Vis and (b) CD spectra of the system Ni(II)/-LAHYNK- at various pH values.



Scheme 3: Possible structure of $NiH_{-1}L$.

Following the above conclusions, a possible coordination proposal for species $CuH_{-2}L$ is presented in Scheme 2. The metal ion coordination sphere includes the imidazole and three-deprotonated amide nitrogens, (the last belonging to the acetamido-group) forming three stable, six- and five-membered chelate rings.

Two more base consuming processes are observed in the pH range 10.5–12.0 corresponding to the formation of complexes $CuH_{-3}L$ and $CuH_{-4}L$. Spectroscopic parameters (Table 4) for these species are almost identical to the ones of $CuH_{-2}L$, implying the same coordination mode $\{1N_{im}, 3N_{amide}\}$. The pK_a value of the ionization reaction leading to $CuH_{-3}L$ is 10.53, exactly the same to that of the ε-NH_3 group of Lys residue in the free ligand (Table 2). For the last dissociable proton (species $CuH_{-4}L$), a pK_a value of 11.65 was calculated. An axially coordinated water molecule may be the best candidate of this additional proton (hydrolysis). In that case, a small red shift in the UV-Vis spectra and the decrease of the $A_{||}$ (EPR) is expected [16, 32]. Unfortunately, the spectroscopic data (Table 4, Figure 2) provide no evidence on that. The only reasonable explanation remaining is the ionization and not coordination of the pyrrole N(1) of the imidazole ring. It is known [33] that the acidity of the pyrrole nitrogen increases upon coordination of a metal ion at the (N3) nitrogen atom. In the case of Cu(II):GlyGlyHis, a pK_a value of about 10.7 was calculated [34] while even lower values are observed when a second metal ion coordinates to this group. pK_a value of about 9.6 has been measured in the case of GlyHis:Cu(II) interaction and a tetranuclear structure with bridging imidazole was proposed [35]. Our value 11.65 is comparable to that corresponding to pyrrole ionization (and not coordination) in the system Cu(II):His 1 : 2 (11.7) [36] and Pd(GlyGlyHis-H_{-2}) complex (11.3) [37]. To our knowledge, this is the first reported protected peptide presenting this type of ionization.

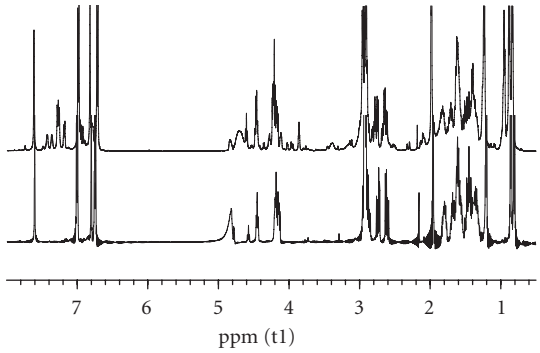


FIGURE 5: ^{1}H-NMR spectra (overlaid) of the free peptide (bottom) and of the Ni(II)-peptide solution (1 : 2) in D_2O at pH* = 9.5.

3.3. Ni(II) complexes

The potentiometric study of the system Ni(II)-LAHYNK was performed in a 1 : 2 molar ratio in the pH range 4–11, in order to achieve shorter equilibrium times especially in neutral and alkaline pH values where sluggish reactions involving kinetically inert Ni(II) low-spin complexes are expected. The species distribution diagram is presented in Figure 3 while calculated stability data have already been presented in Table 3.

The UV/Vis and CD spectra and the spectroscopic parameters at the pH range 4–12 at a metal:peptide ratio 1 : 2 are presented in Figure 4 and Table 4, respectively.

In general, the coordination properties of -LAHYNK- toward Ni(II) ions resemble the typical binding motifs of Ni(II) complexes with several peptides containing only one His residue in an internal position in their sequence [4, 10, 15, 38]. Five nickel complexes were detected in the pH range 4–12, namely NiH_2L, NiHL, $NiH_{-1}L$, $NiH_{-2}L$, and $NiH_{-3}L$.

The complex NiH_2L is initially formed in the pH range 4.0–9.5 with an imidazole monodentate binding in a distorted octahedral geometry [10, 11, 39]. The log K value (3.21) for the reaction Ni(II) + $H_2L \leftrightarrow NiH_2L$ shows a good agreement with literature values corresponding to monodentate coordination of imidazole N-donors (Ac-FKHV-NH_2: 2.92, Ac-AKRHRK-NH_2: 2.92, Ac-TESHAK-am: 3.20, Ac-ELAKHA:3.55) [4, 7, 11, 25].

On increasing pH, species NiH_2L release a proton with a pK_a of 8.61. The pK_a value at 8.61 for the deprotonation process $NiH_2L \rightarrow NiHL + H^+$ is comparable to the values for peptides with amide nitrogen coordination [10, 11, 15, 40]. The resultant complexes NiL cannot be detected spectroscopically due to overlap with other species in the pH range 7–9.5 and their low concentration. However, it is possible to characterize them on the basis of the stability constants corrected for protonation log K^* [15]. The log K^* value of these species (−11.63) are within the data range (−11.42−11.98) of similar complexes adopting an $\{N_{im}, N_{amide}\}$ binding mode [4, 8, 23], supporting the coordination of Ni(II) ions through the imidazole and the amide nitrogen of the His residue forming a stable six-membered chelate ring.

The next three species $NiH_{-1}L$, $NiH_{-2}L$, and $NiH_{-3}L$ are detected above pH 8. The first one $NiH_{-1}L$ is derived from NiHL upon simultaneous release of two amide protons with an average pK_a value of 8.63 (Table 3). This process is very common in coordination chemistry of Ni(II)-peptides and the driving force for this is the formation of a stable square planar low-spin diamagnetic Ni(II) complex [15]. The data for $NiH_{-1}L$, $NiH_{-2}L$, and $NiH_{-3}L$ in Table 4 (d-d bands centered at λ_{max} = 422 − 446 nm, the charge transfe bands $N_{im} \rightarrow$ Ni(II), and $N_{amide} \rightarrow$ Ni(II) detected at 329–336 nm) suggest the cooperative deprotonation and subsequent coordination of two more amide donors [7, 10, 11] saturating the equatorial plane of the Ni(II) ion in $NiH_{-1}L$ (Scheme 3). To strengthen more our binding proposal, the high-resolution ^{1}H-NMR of the -LAHYNK-:Ni(II) system in 2 : 1 molar ratio was recorded in D_2O at pH* = 9.5, providing an indirect proof of existence of the diamagnetic complex (Figure 5). Selected chemical shifts (δ, ppm) and chemical shifts differences ($\Delta\delta$, ppm) between free and Ni(II)-complexed peptide have already been presented in Table 1. Large chemical shifts differences are clearly observed for the α-H of the -L-A-H part of our peptide and of the C(2) –H of the imidazole ring confirming the proposed coordination mode.

Finally, the last two base-consuming processes which correspond to the formation of $NiH_{-2}L$ and $NiH_{-3}L$ species (pK_a values 9.75 and 10.74, resp.) are assigned to deprotonation (and no coordination) of Tyr –OH and Lys ε-NH_3^+ groups in the complexed peptide based on the similarity of the pK_a data for the free ligand (pK_a 9.52 and 10.53, resp.).

4. CONCLUSIONS

In this paper, the coordination properties of the N- and C-terminal blocked hexapeptide Ac-Leu-Ala-His-Tyr-Asn-Lys-amide (LAHYNK) representing the 80–85 fragment of histone H2B towards Cu(II) and Ni(II) have been investigated. Potentiometric and spectroscopic studies were used to establish the stoichiometry, stability and possible structures of all species formed in aqueous solutions.

In the case of Cu(II) complexes, it was found that at low pH values the initial binding site is the histidyl imidazole (CuH_2L). The next species CuL formed at physiological pH values is a 3N complex $\{N_{im}, 2N_{amide}\}$. Its high thermodynamic stability is reflected in the cooperativity of the two amide ionization reactions and the suppression of the third leading to 4N species. The almost exclusive formation of the predominant species CuL in neutral media which accounts for almost 98% of the total metal ion concentration at pH 7.3 may imply that at pH values accessible by biological systems the sequence -LAHYNK- of histone H2B provides very efficient binding sites for metal ion. In more basic solutions, a third proton is titrated (pK_a = 9.04) and species with stoichiometry $CuH_{-1}L$ are formed. The coordination proposal for these is the same $\{N_{im}, 2N_{amide}\}$ differing only in the deprotonated but uncoordinated phenolic –OH group. Finally, above pH ∼ 10, 4N$\{1N_{im}, 3N_{amide}\}$ species are detected in which acetamido-nitrogen donor saturates the equatorial plane of Cu(II). In the following two complexes

$CuH_{-3}L$ and $CuH_{-4}L$, a $\{1N_{im}, 3N_{amide}\}$ binding is also evident following our experimental data, differing only in the deprotonated but uncoordinated phenolic –OH and pyrrole N(1) groups, respectively. It should be mentioned that in our knowledge, this is the first report of a protected peptide presenting this kind of ionization.

The coordination properties of -LAHYNK- toward Ni(II) ions resemble the typical binding motifs of Ni(II) complexes with several peptides containing only one His residue in an internal position in their sequence, that is, the monodentate imidazole binding in species NiH_2L and the formation of typical low-spin diamagnetic $4N\{1N_{im}, 3N_{amide}\}$ complexes above pH ∼ 8 (species $NiH_{-n}L$, $n = 1 - 3$) (Scheme 3).

No hydrolysis of the peptide was observed upon coordination to both Ni(II) and Cu(II) metal ions, in accordance to the same proposed mechanism of our group [10, 14] and others [9, 12] for such a process, implying the presence of a Ser- or Thr-containing an –OH group near the coordination sites [10, 14] to be important for the hydrolytic process. The formation of a 4N planar Ni(II) complex reported to be an important factor as well [9] is also present in our system.

REFERENCES

[1] K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, and T. J. Richmond, "Crystal structure of the nucleosome core particle at 2.8 Å resolution," *Nature*, vol. 389, no. 6648, pp. 251–260, 1997.

[2] W. Bal, R. Liang, J. Lukszo, S.-H. Lee, M. Dizdaroglu, and K. S. Kasprzak, "Ni(II) specifically cleaves the C-terminal tail of the major variant of histone H2A and forms an oxidative damage-mediating complex with the cleaved-off octapeptide," *Chemical Research in Toxicology*, vol. 13, no. 7, pp. 616–624, 2000.

[3] W. Bal, J. Lukszo, and K. S. Kasprzak, "Interactions of nickel(II) with histones: enhancement of 2′-deoxyguanosine oxidation by Ni(II) complexes with CH_3CO-Cys-Ala-lle-His-NH_2, a putative metal binding sequence of histone H3," *Chemical Research in Toxicology*, vol. 9, no. 2, pp. 535–540, 1996.

[4] M. A. Zoroddu, M. Peana, T. Kowalik-Jankowska, H. Kozlowski, and M. Costa, "The binding of Ni(II) and Cu(II) with the N-terminal tail of the histone H4," *Journal of the Chemical Society, Dalton Transactions*, no. 3, pp. 458–465, 2002.

[5] M. A. Zoroddu, M. Peana, and S. Medici, "Multidimensional NMR spectroscopy for the study of histone H4-Ni(II) interaction," *Dalton Transactions*, no. 3, pp. 379–384, 2007.

[6] M. Mylonas, G. Malandrinos, J. C. Plakatouras, et al., "Stray Cu(II) may cause oxidative damage when coordinated to the -TESHHK- sequence derived from the C-terminal tail of histone H2A," *Chemical Research in Toxicology*, vol. 14, no. 9, pp. 1177–1183, 2001.

[7] M. Mylonas, J. C. Plakatouras, N. Hadjiliadis, A. Krężel, and W. Bal, "Potentiometric and spectroscopic studies of the interaction of Cu(II) ions with the hexapeptides AcThrAlaSerHisHisLysNH$_2$, AcThrGluAlaHisHisLysNH$_2$, AcThrGluSerAlaHisLysNH$_2$ and AcThrGluSerHisAlaLysNH$_2$, models of C-terminal tail of histone H2A," *Inorganica Chimica Acta*, vol. 339, pp. 60–70, 2002.

[8] M. Mylonas, A. Krężel, J. C. Plakatouras, N. Hadjiliadis, and W. Bal, "The binding of Ni(II) ions to terminally blocked hexapeptides derived from the metal binding -ESHH- motif of histone H2A," *Journal of the Chemical Society, Dalton Transactions*, no. 22, pp. 4296–4306, 2002.

[9] A. Krężel, M. Mylonas, E. Kopera, and W. Bal, "Sequence-specific Ni(II)-dependent peptide bond hydrolysis in a peptide containing threonine and histidine residues," *Acta Biochimica Polonica*, vol. 53, no. 4, pp. 721–727, 2006.

[10] M. Mylonas, J. C. Plakatouras, and N. Hadjiliadis, "Interactions of Ni(II) and Cu(II) ions with the hydrolysis products of the C-terminal -ESHH- motif of histone H2A model peptides. Association of the stability of the complexes formed with the cleavage of the -E-S- bond," *Dalton Transactions*, no. 24, pp. 4152–4160, 2004.

[11] T. Karavelas, M. Mylonas, G. Malandrinos, et al., "Coordination properties of Cu(II) and Ni(II) ions towards the C-terminal peptide fragment -ELAKHA- of histone H2B," *Journal of Inorganic Biochemistry*, vol. 99, no. 2, pp. 606–615, 2005.

[12] W. Bal, J. Lukszo, K. Bialkowski, and K. S. Kasprzak, "Interactions of Nickel(II) with histones: interactions of Nickel(II) with CH_3CO-Thr-Glu-Ser-His-His-Lys-NH_2, a peptide modeling the potential metal binding site in the "C-tail" region of histone H2A," *Chemical Research in Toxicology*, vol. 11, no. 9, pp. 1014–1023, 1998.

[13] M. Mylonas, A. Krężel, J. C. Plakatouras, N. Hadjiliadis, and W. Bal, "Interactions of transition metal ions with His-containing peptide models of histone H2A," *Journal of Molecular Liquids*, vol. 118, no. 1–3, pp. 119–129, 2005.

[14] T. Karavelas, G. Malandrinos, N. Hadjiliadis, et al., "Coordination properties of Cu(II) and Ni(II) ions towards the C-terminal peptide fragment -TYTEHA- of histone H4," *Dalton Transactions*, pp. 1215–1223, 2008.

[15] H. Kozlowski, W. Bal, M. Dyba, and T. Kowalik-Jankowska, "Specific structure-stability relations in metallopeptides," *Coordination Chemistry Reviews*, vol. 184, no. 1, pp. 319–346, 1999.

[16] H. Sigel and R. B. Martin, "Coordinating properties of the amide bond. Stability and structure of metal ion complexes of peptides and related ligands," *Chemical Reviews*, vol. 82, no. 4, pp. 385–426, 1982.

[17] H. Kozlowski, T. Kowalik-Jankowska, and M. Jeżowska-Bojczuk, "Chemical and biological aspects of Cu^{2+} interactions with peptides and aminoglycosides," *Coordination Chemistry Reviews*, vol. 249, no. 21-22, pp. 2323–2334, 2005.

[18] I. Sóvág ó and K. Ősz, "Metal ion selectivity of oligopeptides," *Dalton Transactions*, no. 32, pp. 3841–3854, 2006.

[19] M. W. Pennington and B. M. Dunn, *Peptide Synthesis Protocols*, vol. 35 of *Methods in Molecular Biology*, Humana Press, Totowa, NJ, USA, 1994.

[20] G. A. Grant, *Synthetic Peptides. A User's Guide*, W. H. Freeman, New York, NY, USA, 1992.

[21] H. Irving, M. G. Miles, and L. D. Pettit, "A study of some problems in determining the stoichiometric proton dissociation constants of complexes by potentiometric titrations using a glass electrode," *Analytica Chimica Acta*, vol. 38, no. 4, pp. 475–488, 1967.

[22] P. Gans, A. Sabatini, and A. Vacca, "Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite of programs," *Talanta*, vol. 43, no. 10, pp. 1739–1753, 1996.

[23] V. Jószai, Z. Nagy, K. Ősz, et al., "Transition metal complexes of terminally protected peptides containing histidyl residues," *Journal of Inorganic Biochemistry*, vol. 100, no. 8, pp. 1399–1409, 2006.

[24] B. Decock-Le Reverend, F. Liman, C. Livera, L. D. Pettit, S. Pyburn, and H. Kozlowski, "A potentiometric and spectroscopic study of the interaction of angiotensin II and some of its peptide fragments with copper(II)," *Journal of the Chemical Society, Dalton Transactions*, no. 4, pp. 887–894, 1988.

[25] B. Belosi, E. Gaggelli, R. Guerrini, et al., "Copper binding to the neurotoxic peptide $PrP_{106\text{-}126}$: thermodynamic and structural studies," *ChemBioChem*, vol. 5, no. 3, pp. 349–359, 2004.

[26] S. Van Doorslaer, G. M. Cereghetti, R. Glockshuber, and A. Schweiger, "Unraveling the Cu^{2+} binding sites in the C-terminal domain of the murine prion protein: a pulse EPR and ENDOR study," *Journal of Physical Chemistry B*, vol. 105, no. 8, pp. 1631–1639, 2001.

[27] B. Bóka, A. Myari, I. Sóvágó, and N. Hadjiliadis, "Copper(II) and zinc(II) complexes of the peptides Ac-HisValHis-NH_2 and Ac-HisValGlyAsp-NH_2 related to the active site of the enzyme CuZnSOD," *Journal of Inorganic Biochemistry*, vol. 98, no. 1, pp. 113–122, 2004.

[28] M. A. Zoroddu, T. Kowalik-Jankowska, H. Kozlowski, et al., "Interaction of Ni(II) and Cu(II) with a metal binding sequence of histone H4: AKRHRK, a model of the H4 tail," *Biochimica et Biophysica Acta*, vol. 1475, no. 2, pp. 163–168, 2000.

[29] T. Kowalik-Jankowska, M. Ruta-Dolejsz, K. Wiśniewska, L. Łankiewicz, and H. Kozlowski, "Copper(ll) complexation by human and mouse fragments (11–16) of β-amyloid peptide," *Journal of the Chemical Society, Dalton Transactions*, no. 24, pp. 4511–4519, 2000.

[30] M. Łuczkowski, H. Kozlowski, M. Stawikowski, et al., "Is the monomeric prion octapeptide repeat PHGGGWGQ a specific ligand for Cu^{2+} ions?" *Journal of the Chemical Society, Dalton Transactions*, no. 11, pp. 2269–2274, 2002.

[31] M. Orfei, M. C. Alcaro, G. Marcon, et al., "Modeling of copper(II) sites in proteins based on histidyl and glycyl residues," *Journal of Inorganic Biochemistry*, vol. 97, no. 3, pp. 299–307, 2003.

[32] I. Sóvágó, D. Sanna, A. Dessí, K. Várnagy, and G. Micera, "EPR and potentiometric reinvestigation of copper(II) complexation with simple oligopeptides and related compounds," *Journal of Inorganic Biochemistry*, vol. 63, no. 2, pp. 99–117, 1996.

[33] R. B. Martin, "Complexes of a-amino acids with chelatable side chain donor atoms," *Metal Ions in Biological Systems*, vol. 9, pp. 1–39, 1979.

[34] H. Aiba, A. Yokoyama, and H. Tanaka, "Copper(II) complexes of glycyl-L-histidine, glycyl-L-histidylglycine, and glycylglycyl-L-histidine in aqueous solution," *Bulletin of the Chemical Society of Japan*, vol. 47, no. 6, pp. 1437–1441, 1974.

[35] P. J. Morris and R. B. Martin, "Tetramer formation in tetragonal transition metal ion complexes of glycyl-*L*-histidine," *Journal of Inorganic and Nuclear Chemistry*, vol. 33, no. 9, pp. 2913–2918, 1971.

[36] P. J. Morris and R. B. Martin, "Tetrahedral complexes of cobalt(II) with L-histidine, histamine, imidazole, and N – Acetyl$_{-L-}$histidine," *Journal of the American Chemical Society*, vol. 92, no. 6, pp. 1543–1546, 1970.

[37] T. P. Pitner, E. W. Wilson Jr., and R. B. Martin, "Properties of palladium(II) complexes of peptides and histidine in basic solutions," *Inorganic Chemistry*, vol. 11, no. 4, pp. 738–742, 1972.

[38] T. Kowalik-Jankowska, M. Ruta-Dolejsz, K. Wiśniewska, and L. Łankiewicz, "Cu(II) interaction with N-terminal fragments of human and mouse β-amyloid peptide," *Journal of Inorganic Biochemistry*, vol. 86, no. 2-3, pp. 535–545, 2001.

[39] M. A. Zoroddu, T. Kowalik-Jankowska, H. Kozlowski, K. Salnikow, and M. Costa, "Ni(II) and Cu(II) binding with a 14-amino acid sequence of Cap43 protein, TRSRSHTSEGTRSR," *Journal of Inorganic Biochemistry*, vol. 84, no. 1-2, pp. 47–54, 2001.

[40] W. Bal, H. Kozlowski, R. Robbins, and L. D. Pettit, "Competition between the terminal amino and imidazole nitrogen donors for coordination to Ni(II) ions in oligopeptides," *Inorganica Chimica Acta*, vol. 231, no. 1-2, pp. 7–12, 1995.