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Effect of Threonine on the Interaction Between Tau Protein Fragments and M^{2+} Ions ($M = Ni, Cu, Zn$)

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ABSTRACT

Systematic studies of metal complexes of tau protein fragments may contribute to gaining more information about its role in the neurodegenerative tauopathies. Previous studies have confirmed that the tau fragment containing the amino acid His32 has a higher metal binding affinity than other fragments containing histidine, due to the presence of a threonine near the histidine. In order to investigate the role of threonine in more detail, the Cu(II), Zn(II), and Ni(II) complexes of the tau(371–376) fragment (Ac-IETHKL-NH₂) and three tau(29–34) mutants (Ac-ATAHQD-NH₂, Ac-ATMHQD-NH₂, Ac-AAMHQD-NH₂) were examined. The presences of -TXH- moiety resulted in a small increase in the stability of the imidazole coordinated complexes as well as an observable change in the CD and UV-Vis spectra for copper(II) and nickel(II) complexes. On the other hand, the binding of nickel(II) ion to the -TXH- part of the molecule may induce hydrolytic processes in peptides. The hydrolytic process was studied in the presence of nickel(II) under different condition in peptides containing -TXH- and -XTH- moiety. The Ni(II)-induced hydrolytic process was obvious in the case of peptide containing -TXH- sequence in alkaline medium. However, the tau(371–376) fragment containing -XTH- sequence did not show any hydrolytic activity in the presence of Ni(II).

1 | Introduction

Tau protein has a significant role in the human brain, because it stabilizes the neuronal microtubules under normal physiological conditions [1]. However, in the context of incomprehensible pathological mechanisms, tau protein undergoes structural modification primarily via the process of hyperphosphorylation, which is induced by kinase enzymes. This process ultimately results in the destruction of the protein, the accumulation of aberrant toxic aggregates, and the subsequent generation of neurofibrillary tangles [2]. These insoluble neurofibrillary tangles are characterized by their high toxicity, which contributes to the selective destruction of neurons within the human brain. This process has the potential to initiate the development of

neurodegenerative disorders, such as Alzheimer's disease [3, 4]. The involvement of metal ions in neurodegenerative diseases has been demonstrated by numerous analytical measurements. Both essential and toxic metal ions have been identified in significant quantities in plaques, which are characterized by the aggregation process of proteins [5–7]. The extent of protein aggregation is influenced by both the concentration and the oxidation state of the metal ions. Furthermore, the role of specific metal ions, such as copper or iron, in catalyzing biological redox reactions has been documented [8–11].

The sequence of tau protein consists of 441 amino acid residues, including 12 histidyl and 2 cysteinyl residues, which are effective binding sites to coordinate metal ions. Other polar side chains

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like glutamyl, aspartyl, seryl, and threonyl greatly improve this type of interaction. The coordinating behavior of tau protein as a biological ligand is much less studied as compared to those of amyloid or prion peptides. Some previous literature studies have focused on the R-regions of the protein, which are the microtubule-binding domains of the protein [12, 13], also there are some publications connected to the biological role of the N-terminal part of the protein[14]. Numerous publications have addressed the metal-binding ability of amyloid-beta and prion proteins and their various peptide fragments, as well as their connection to neurodegeneration. The results of these studies strongly boost the inclination of these proteins toward various metal ions [15–17]. These studies proved that imidazole-N donor atoms of these biological ligands are the primary metal binding sites for different metal ions. Since these side chains may be primary binding sites for essential copper(II) and zinc(II), as well as nonbeneficial nickel(II) ions, previously we performed the systematic studies of copper(II), zinc(II), and nickel(II) complexes of fragments containing histidine and cysteine in different amino acid environments [18–21]. Nickel(II) is not an essential element for the human body, but in peptide chemistry, it has a similar coordination chemistry behavior to that of copper(II). Under normal physiological conditions, the body is capable of naturally excreting nickel(II) ions. The *Helicobacter pylori* bacterium is the sole organism known to contain this metal ion in its natural environment and to be present in the human stomach during infections [22, 23]. However, under specific genetic abnormalities or in the presence of excessive environmental stress, an accumulation of nickel(II) metal ions can be suspected, which may result in elevated nickel(II) metal ion concentrations. Nickel(II) was also involved in our study, as a comprehensive review showed elevated concentrations of nickel(II) metal ions in biological samples obtained from patients with confirmed neurodegenerative diseases from different geographical locations [24].

These studies reveal that the histidine at position 32 (tau(29–33) fragment) binds copper(II) and nickel(II) ions with greater affinity than the fragment containing histidine at position 14 (tau(9–16)) [18, 20]. The various potentiometric and spectroscopic experiments confirmed that threonine, located by one amino acid away from histidine in the terminally protected peptides, also contributes to the increased stability of copper(II) and nickel(II) complexes.

Moreover, the presence of –ThrXaaHis– sequence in the molecule has effect on the hydrolytic processes in the presence of nickel(II) ion. The Ni(II) ions-induced hydrolysis of amide bond of peptides has been studied for some designed and native protein fragments. These studies have shown that Ni(II)-induced hydrolysis of the peptide bond happens quickly in the protein sequence –Z₁aaThr(Ser)XaaHisZ₂aa– (–Z₁T(S)XHZ₂–) (where Z₁aa/Z₁ and Z₂aa/Z₂ represent any amino acid residue and Xaa/X represents any amino acid residue except proline). The position of the hydrolyzed bond is between the Z₁ and threonyl or seryl residue [25, 26]. One of the key conditions for initiating the Ni(II)-induced hydrolytic process is the formation of a square planar Ni(II) complex, whose coordination mode includes the imidazole-N donor and three amide nitrogen atoms preceding histidine. This complex formation process, however, requires slightly basic conditions, it sterically bends the backbone of the peptide sequence, bringing the strong hydroxyl nucleophile of the threonyl or seryl residue close to the previous amide bond.

This changes the type of coordination modes around Ni(II) through molecular rearrangement and forms an ester bond through the peptide molecule, which can be easily hydrolyzed in the presence of a base [27]. There are important factors that can affect the rate of the hydrolytic process in the presence of Ni(II) ion, including temperature, pH of the reaction medium, time, and type of amino acid residue near the potential hydrolysis site. It was found that huge hydrophobic side chains adjacent to the histidine residue promote a fast reaction [28, 29].

Some studies of Ni(II)-induced hydrolysis were performed at high temperatures (45°C–50°C) and in the pH range of 8.2–8.5 [27]. However, some studies showed that nickel(II) promotes fast hydrolytic activity even at lower temperatures [30]. In our recently published articles, the hydrolytic process was studied in the presence of Ni(II) ion for different peptides at 25°C and 37°C, pH = 7.4 and pH = 8.2 [31, 32].

The studies summarized here focus on the effect of threonine near histidine on complex formation and hydrolytic processes, through the investigation of copper(II), nickel(II), and zinc(II) complexes of two tau fragments or their mutants. In the case of the tau mutant, we systematically altered the amino acid sequence in order to obtain a more accurate picture of the role of threonine, which is close to histidine. The peptides studied are the native tau(371–376) fragment (Ac-IETHKL-NH₂) from the R-domain and mutants of the tau(29–34) fragment (tau(Y29A-M31A-34): Ac-ATAHQD-NH₂, tau(Y29A-34): Ac-ATMHQD-NH₂ and tau(Y29A-T30A-34): Ac-AAMHQD-NH₂) from the N-terminal region. The structural formulae of studied peptides are presented in Figure 1. In the first step, we analyzed the effect of threonine on

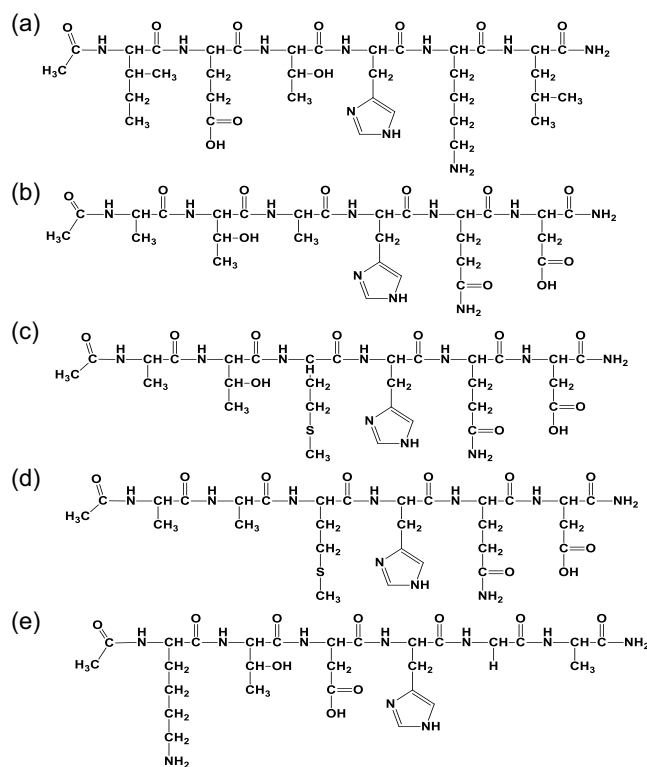


FIGURE 1 | Chemical structure of the studied ligands: (a) Ac-IETHKL-NH₂, (b) Ac-ATAHQD-NH₂, (c) Ac-ATMHQD-NH₂, (d) Ac-AAMHQD-NH₂, and (e) Ac-KTDHGA-NH₂.

the stability, on ultraviolet-visible (UV-Vis) and circular dichroism (CD) spectroscopic data of the complexes. Subsequently, we studied the hydrolytic processes of tau(385–390) (Ac-KTDHGA-NH₂), Ac-ATAHQD-NH₂, and Ac-IETHKL-NH₂ at 25°C and at two different pH values in the presence of nickel(II) ions to determine the role of threonine present in different locations on the hydrolytic reactions.

2 | Results and Discussion

2.1 | Acid-Base Properties of the Peptides

The protonation constants of the peptide ligands were determined by potentiometric measurements, and the values for the peptides are listed in Table 1. All peptides are protected from both N and C-terminus and contain one histidyl residue, which acts as a primary anchor for metal ion in solution. The carboxylate groups of glutamyl and aspartyl as well as the lysyl residues participate in the equilibrium processes for these ligands. The stability constants and the corresponding pK values for these ligands are listed in Table 1, in which three of these peptides contain two deprotonation sites belonging to histidyl and aspartyl residues, while one peptide fragment possesses three deprotonation sites, including histidyl, glutamyl, and lysyl residues.

The pK values of imidazole-N donors in the absence of any other side chains are usually in between 6 and 6.3 [33], but for all these studied systems, the imidazole pK values are slightly higher due to the presence of glutamic or aspartic acid residues. The carboxylic groups of these side chains are considered to form weak hydrogen bonds with the imidazole-N atom and hinder its deprotonation process [21]. The deprotonation of the carboxylic groups of glutamyl or aspartyl moiety occurs in slightly acidic solution, where the pH value is below 4.10. Because of the high basicity of the lysyl side chain, it has a high pK value, which is characteristic of tau(371–376) and tau(385–390) fragments.

2.2 | Copper(II) Complexes of the Peptides

The stability constants of the copper(II) complexes were obtained by potentiometric titrations, and the data are included in Table 2. In peptides, imidazole nitrogen behaves as the anchor group; above pH 4, complexes with imidazole coordination are formed. The distribution curves of equimolar solution of copper(II) and peptides are plotted in Figure 2a,b. For tau(371–376), the Cu(II)-N_{im} coordinated complex is [CuHL], in which lysyl residue still protonated. The imidazole coordinated species are present in the acidic medium with moderate percentage and always occur simultaneously with either free copper(II) ions or other complexes. The imidazole coordinated complexes are

TABLE 1 | Formation (logβ) and deprotonation constants (pK) values of tau fragments and their mutants (T = 298 K, I = 0.2 M KCl).

Species	Ac-IETHKL-NH ₂ tau(371–376)	Ac-AAMHQD-NH ₂ tau(Y29A-T30A-34)	Ac-ATMHQD-NH ₂ tau(Y29A-34)	Ac-ATAHQD-NH ₂ tau(Y29A-M31A-34)	Ac-KTDHGA-NH ₂ tau(385–390) [21]
HL	10.33(5)	6.55(5)	6.51(1)	6.53(1)	10.34
H ₂ L	16.72(7)	10.27(1)	10.27(1)	10.23(3)	16.85
H ₃ L	20.78(1)	—	—	—	20.41
pK(Asp)	—	3.72	3.76	3.70	3.56
pK(Glu)	4.06	—	—	—	—
pK(His)	6.39	6.55	6.51	6.53	6.51
pK(Lys)	10.33	—	—	—	10.34

TABLE 2 | The stability constants (logβ) of the Cu(II) complexes of tau(371–376) and tau mutants (T = 298 K, I = 0.2 M KCl).

Species	Ac-IETHKL-NH ₂ tau(371–376)	Ac-AAMHQD-NH ₂ tau(Y29A-T30A-34)	Ac-ATMHQD-NH ₂ tau(Y29A-34)	Ac-ATAHQD-NH ₂ tau(Y29A-M31A-34)	Ac-KTDHGA-NH ₂ tau(385–390) [21]
[CuHL]	14.56(2)	—	—	—	14.43
[CuL]	—	5.17(5)	5.09(2)	5.28(6)	—
[CuH ₁ L]	2.15(2)	—	—	—	3.41
[CuH ₂ L]	−6.57(2)	−7.43(5)	−6.02(2)	−6.01(5)	−5.28
[CuH ₃ L]	−16.47(2)	−15.79(8)	−14.47(3)	−14.77(8)	−15.28
pK ₁₂ (amide)	6.21	6.30	5.56	5.65	5.51
pK ₃ (amide)	8.72	8.36	8.45	8.76	8.65
pK(Lys-NH ₃ ⁺)	9.9	—	—	—	10.00
logK(Cu–N _{im})	4.23	5.17	5.09	5.28	4.09

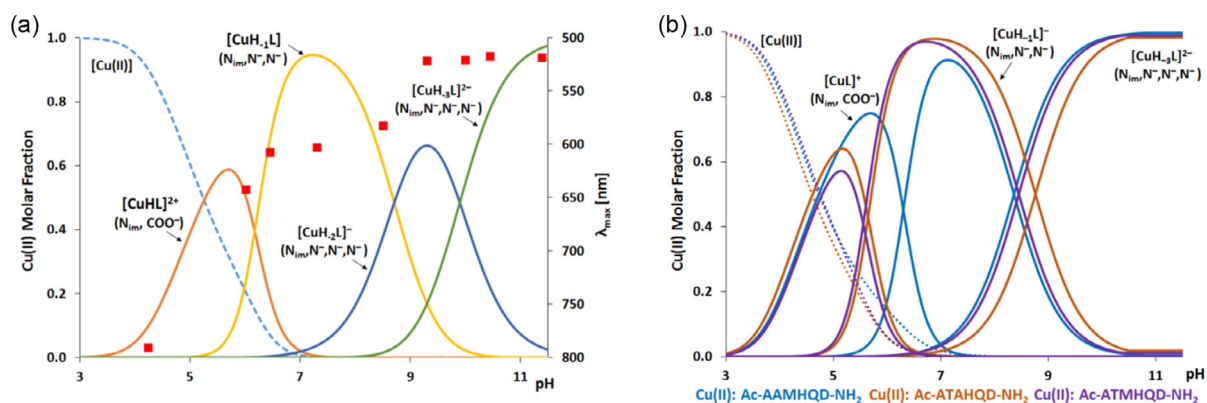


FIGURE 2 | Metal ion distribution curves in the (a) copper(II): tau(371–376) system with the change in absorption maxima (marked with red squares) ($c(L) = c(M) = 1.6$ mM) and (b) copper(II): tau(29–34) mutants systems ($c(L) = c(M) = 1.0$ mM).

characterized by the $\log K(M-N_{im})$ value (see (Equation 3)). This value is around 3.5 for Cu(II) complexes of peptides containing one histidine (e.g. $\log K(M-N_{im})$ 3.60 for Ac-FKHV-NH₂ [33], 3.50 for Ac-LAHYNK-NH₂ [34]), but the interaction of amino acid side chains (Asp, Glu, Met, Thr, Ser) close to histidine with the copper(II) ion may result in a slight increase in complex stability (e.g. $\log K(M-N_{im})$ 4.96 for Ac-TYTEHA-NH₂ [35], 4.47 for Ac-TESAHK-NH₂ [36], and 4.51 for Ac-TESHAK-NH₂ [36]). This can also be observed in the case of the present studied peptides.

Based on potentiometric data, only mononuclear species can be observed in the studied systems, and there is no formation of any type of bis(ligand) complexes, which is due to the steric effect of side chains and the preference of deprotonation and coordination of peptide amide bond.

The deprotonation and metal ion coordination of the first two amide bond functions take place in a cooperative manner for tau(385–390), tau(371–376), and also for the tau(29–34) mutants forming $[CuH_2LH]=[CuH_2L]$ and $[CuH_2L]$ species, respectively, which were the main complex species at physiological pH. This is characteristic of all N-terminally protected peptides that contain histidine [16]. In the case of the tau(385–390) [21] and tau(371–376) peptides, the deprotonation of the third amide nitrogen occurred above pH 8.5 to form $[CuH_3LH]=[CuH_2L]$ complex, which is followed by the deprotonation of the lysine ammonium group, resulting in the formation of $[CuH_3L]$ species at pH above 9.5 (see Figure 2a).

The wavelength of visible absorption maxima detected in the solution of copper(II): tau(371–376) are plotted in Figure 2a,

and it is clear that the change of the coordination modes from (N^-, N^-, N_{im}) -coordinated species to (N^-, N^-, N^-, N_{im}) -coordinated species is accompanied by a significant blueshift of the absorption maxima (Figure S1, Supporting Information in supplementary material). For the peptides Ac-ATMHQD-NH₂ and Ac-ATAHQD-NH₂, the absorption maximum characteristic of the (N^-, N^-, N^-, N_{im}) -coordinated complex appears at higher wavelength than that of the other peptides (Table 3). A slight redshift in the absorption maximum usually indicates an axial interaction in the copper(II) complex [37, 38]. Thus, in the case of the peptides studied, the weak axial coordination of the Thr-OH group in the -TXH- sequence may cause the different spectral parameters.

The spectral data of circular dichroism (CD) measurement for the studied hexapeptides are collected in Table S1, Supporting Information, and illustrated in Figure S2, Supporting Information. CD spectra can be detected in slightly acidic pH in parallel with the formation of amide coordinated complexes. These spectra are plotted in Figure 3a for the (N^-, N^-, N_{im}) -coordinated $[CuH_2LH]$ or $[CuH_2L]$ and Figure 3b for the (N^-, N^-, N^-, N_{im}) -coordinated $[CuH_3L]$ species. These spectra proved that 3N- and 4N-coordinated copper(II) complexes have different spectral characteristics.

It is also obvious from the comparison of CD spectra that the spectrum of copper(II) complexes of Ac-IETHKL-NH₂ and Ac-AAMHQD-NH₂ differ from those of other three peptides, Ac-ATMHQD-NH₂, Ac-ATAHQD-NH₂, and Ac-KTDHGA-NH₂. Since these differences can only be observed when threonine is one amino acid away from histidine in the molecule, this supports the conclusion that only a threonine in given position

TABLE 3 | UV-Vis parameters (λ_{max} [nm] and ϵ [$M^{-1} \cdot cm^{-1}$]) of Cu(II) complexes ($T = 298$ K, $I = 0.2$ M KCl).

Coordination modes	Ac-IETHKL-NH ₂ tau(371–376)	Ac-AAMHQD-NH ₂ tau(Y29A-T30A-34)	Ac-ATMHQD-NH ₂ tau(Y29A-34)	Ac-ATAHQD-NH ₂ tau(Y29A-M31A-34)	Ac-KTDHGA-NH ₂ tau(385–390) [21]
$N_{im} (+COO^-)$	742(36)	718(27)	713(20)	724(25)	750(15) ^a
(N^-, N^-, N_{im})	603(94)	601(74)	592(44)	593(55)	588(77)
(N^-, N^-, N^-, N_{im})	519(149)	520(103)	561(52)	561(91)	561(81)

^aThe relatively low concentration of the complex makes the parameter uncertain.

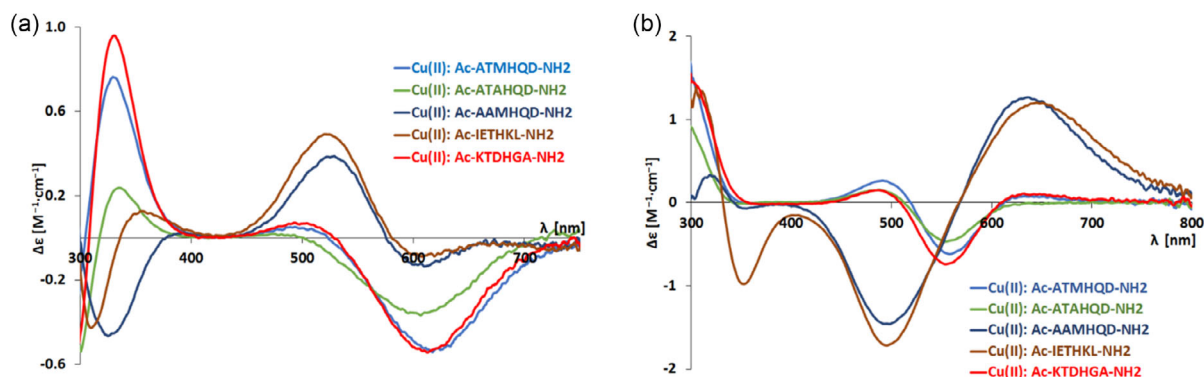


FIGURE 3 | CD spectra of (a) (N^- , N^- , N_{im})-coordinated (3N) complexes at pH around 7.5 and (b) (N^- , N^- , N^- , N_{im})-coordinated (4N) complexes at pH above 10.5 (spectra of tau(385–390) taken from ref. [21]).

causes a change in spectral characteristics. A similar effect has been observed in peptides containing the $-SXH-$ sequence [31].

2.3 | Zinc(II) Complexes of the Peptides

In this study, zinc(II) complexes of the tau(371–376) peptide and another two tau(29–34) mutants have been investigated. Potentiometric measurements of zinc(II)-containing systems showed that zinc(II) has low binding affinity for all three peptides. For the tau(371–376) peptide, the stability constant of the $[ZnHL]$ complex was determined, where zinc(II) is coordinated by the imidazole group and amino group of the lysyl side chain is present in protonated form (Table 4). The formation of a zinc(II)-hydroxide precipitate prevented the determination of any other zinc(II)-peptide species, even in the presence of excess of the ligand. It should be noted that the threonyl residue adjacent to histidine has no effect on the stability of the Zn(II)-imidazole nitrogen species (Table 4, last row).

For Ac-ATMHQD-NH₂ and Ac-ATAHQD-NH₂, the stability constants for imidazole-N-coordinated $[ZnL]$ complexes can be calculated. These values are close to those obtained for other peptides containing a single histidyl residue [20]. In the case of the Ac-ATMHQD-NH₂ peptide, the formation of a mixed hydroxido complex ($[ZnH_{-1}L]$) can also be detected. However, in the case of the Ac-ATAHQD-NH₂ ligand, the zinc(II) hydroxide precipitate formed at a pH of around 6.8, preventing the formation of any hydroxido complex. For the two peptides containing the $-TXH-$ sequence, the stability of the zinc(II)- N_{im} complexes is slightly enhanced by the presence of a threonyl residue.

According to the coordination behavior of zinc(II) ion, it cannot promote the deprotonation of peptide amide bonds, although, this process was reported for the tau(326–333) peptide that involves two vicinal histidyl residues in the R3 domain of the tau protein sequence [20].

2.4 | Nickel(II) Complexes of the Peptides

The nickel(II) complexes of tau(371–376) and three tau(29–34) mutants were studied by potentiometric technique and different spectroscopic tools, including UV-Vis and CD spectroscopic methods. The stability constants of the complexes can be found in Table 5, while the corresponding speciation curves are included in Figure 4. The study of complex formation processes of these systems has shown that only mononuclear complexes are formed.

The Ni(II)- N_{im} coordinated complexes ($[NiHL]$ for tau(371–376) and tau(385–390) [21]; $[NiL]$ for tau(29–34) mutants) are the main species in the physiological pH range. The $\log K(Ni-N_{im})$ values were calculated for all peptides, and these data are very similar to those of other nickel(II) peptide complexes with a monodentate imidazole-N coordination mode [39]. The absorption spectrum recorded below pH 8.5 contains three broad, low-intensity absorption maxima around 270, 390, and 680 nm (Figure S3, Supporting Information). This suggests that Ni(II)- N_{im} coordinated complexes have an octahedral geometry [40].

The nickel(II) ion-induced deprotonation of peptide amide nitrogens takes place above pH 8. In the case of tau(371–376),

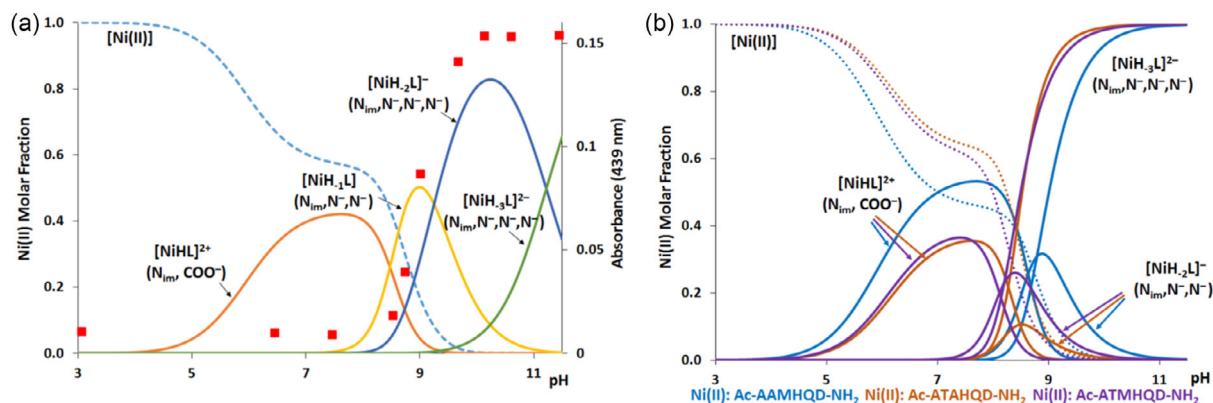
TABLE 4 | The stability constants ($\log \beta$) of the Zn(II) complexes of tau(371–376) and tau mutants (T = 298 K, I = 0.2 M KCl) caption.

Species	Ac-IETHKL-NH ₂ tau(371–376)	Ac-ATMHQD-NH ₂ tau(Y29A-34)	Ac-ATAHQD-NH ₂ tau(Y29A-M31A-34)	Ac-KTDHGA-NH ₂ tau(385–390) [21]
$[ZnHL]$	13.27(3)	—	—	—
$[ZnL]$	—	3.06(2)	3.05(5)	5.05 ^a
$[ZnH_{-1}L]$	—	−4.96(4)	—	—
pK(hydroxido)	—	8.02	—	—
$\log K(Zn-N_{im})$	2.94	3.06	3.05	—

^a $[ZnL] = [Zn(H_{-1})(HL)]$ – mixed hydroxido complex.

TABLE 5 | The stability constants ($\log\beta$) of the Ni(II) complexes of tau(371–376) and tau mutants (T = 298 K, I = 0.2 M KCl).

Species	Ac-IETHKL-	Ac-ATMHQD-			
	NH ₂ tau (371–376)	Ac-AAMHQD-NH ₂ tau(Y29A-T30A-34)	NH ₂ tau (Y29A-34)	Ac-ATAHQD-NH ₂ tau(Y29A-M31A-34)	Ac-KTDHGA-NH ₂ tau(385–390) [21]
[NiHL]	12.99(5)	—	—	—	13.03
[NiL]	—	3.43(5)	3.03(5)	2.98	5.01
[NiH ₁ L]	−4.53(3)	—	—	—	—
[NiH ₂ L]	−13.32(2)	−13.83(5)	−13.20(5)	−13.99	−11.78
[NiH ₃ L]	−23.64(3)	−22.62(3)	−21.40(3)	−21.84	−21.78
pK ₁ (amide)	—	—	—	—	8.02
pK ₁₂ (amide)	8.76	8.63	8.12	8.48	—
pK ₂₃ (amide)	—	—	—	—	8.40
pK ₃ (amide)	8.79	8.79	8.20	7.85	—
pK ₁₂₃ (amide)	8.77	8.68	8.14	8.27	8.27
pK(Lys-NH ₃ ⁺)	10.32	—	—	—	10.00
logK(Ni-N _{im})	2.66	3.43	3.03	2.98	2.69

**FIGURE 4** | Metal ion distribution curves in the (a) nickel(II): tau(371–376) system with the change in absorbance at 439 nm (marked with red squares) ($c(L) = c(M) = 1.6$ mM) and (b) nickel(II): tau(29–34) mutants systems ($c(L) = c(M) = 1.0$ mM).

the lysyl side chain remained protonated during the complex formation process because it does not take part in the metal ion binding [39]. This ammonium group is protonated in all nickel(II) complexes of the peptides containing lysyl residue ($[NiH_2LH] = [NiH_1L]$, $[NiH_3LH] = [NiH_2L]$), and the last deprotonation process ($[NiH_3L]$) corresponds to the deprotonation of the non-bonding lysyl ammonium group.

The pK_{amide} values of the deprotonation reflect that the deprotonation steps occur in a cooperative manner, and the average $pK(amide)$ values of deprotonation of the 3 amide nitrogens ($pK_{123}(amide)$, see (Equation 4)) for different peptides are most relevant for comparison. Therefore, the $(N_{im}, 3N^-)$ coordinated nickel(II) complexes are the major species above pH 9 with $[NiH_3LH]$ and/or $[NiH_3L]$ stoichiometry for the different peptides. Comparison of the $pK_{123}(amide)$ clearly shows that for peptides containing the $-TXH-$ sequence, the deprotonation steps occur at pH about half a pH unit lower, which can be attributed to the presence of the threonine side chain [20, 21].

The UV–Vis spectra reveal that the square planar nickel(II) complexes form around pH 8 with the involvement of amide-N donors in the binding of metal ion. The geometry of complexes is supported by the UV–Vis spectra, in which a peak appears around 450 nm [40] and its intensity increases parallel with the formation of amide and imidazole nitrogens coordinated species (Figures 4a and S3, Supporting Information). On the other hand, the CD active complexes are also detected above pH 8, corresponding to the formation of square planar nickel(II) complexes bound by $((N^-)_x, N_{im})$ donor set (see Figure S4, Supporting Information) [38].

It is also important to note that the pH-dependent changes in the CD absorption spectra are similar for the nickel(II) complexes of tau(371–376) and Ac-AAMHQD-NH₂ peptides, but different from those of peptides containing the $-TXH-$ sequence (Ac-ATMHQD-NH₂, Ac-ATAHQD-NH₂, and Ac-KTDHGA-NH₂). This is illustrated by comparison of CD spectra of different Ni(II)-peptide systems, which are depicted on Figure 5. The CD spectra of tau(371–376) and Ac-AAMHQD-NH₂ peptides show

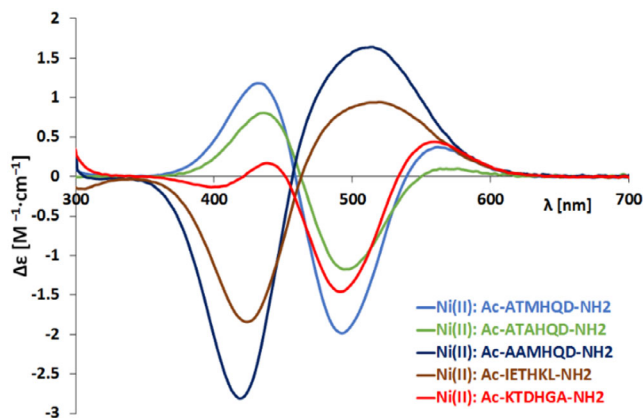


FIGURE 5 | Circular dichroism (CD) spectra of (N⁺,N⁺,N⁺,N_{im})-coordinated (4N) complexes at pH above 10.5 (spectra of tau(385–390) taken from ref. [21]).

intense positive and negative peaks around 510 and 420 nm, respectively, while the CD spectra of the Ni(II) complexes of the other three peptides show a virtually mirror image, with an intense negative peak around 490 nm and a positive peak between 420 and 430 nm. This phenomenon was observed previously in studied similar peptides, and it can be explained by the presence and effect of the threonine –OH group [20, 21, 41].

In addition, the CD spectrum of the latter three peptides changes with time, but this is discussed in more detail in the next paragraph.

2.5 | Hydrolytic Processes of Tau Peptides in the Presence of Nickel(II) Ions

The hydrolytic process occurring in the presence of Ni(II) was investigated in three peptide: tau(385–390) (Ac-KTDHGA-NH₂) fragment, the complex formation processes of which have been described previously [21], tau(371–376) fragment (Ac-IETHKL-NH₂), and one tau(29–34) mutant peptide (Ac-ATAHQD-NH₂). All three peptides contain threonine close to histidine (–TXH– or –XTH– sequence). The hydrolytic reaction was studied at 25°C and at two different pH values: one around eight, close to the physiological pH range, and the other under strongly alkaline conditions, around pH 11.

The hydrolytic process of the Ac-KTDHGA-NH₂ peptide was monitored at two pHs, while the Ac-ATAHQD-NH₂ peptide was monitored at one pH by high performance liquid chromatography (HPLC) for 1 week, and the products were identified by high resolution mass spectrometry (HR-MS) measurements. Figure 6 shows the HPLC chromatograms of Ac-KTDHGA-NH₂ recorded at pH 8 and 11, while Figure S5, Supporting Information, shows the results obtained for the Ac-ATAHQD-NH₂ peptide at pH 8.

The chromatograms of the initial HPLC measurements performed at pH 8 (Figure 6a) showed only the peak of the original peptide with a retention time of 11.1 min. No other process was detected for at least 217 min. As the reaction continues for a longer period of time, two new peaks appear with retention times of 7.9 and 5.1 min, respectively, while the intensity of the ligand peak decreases. A similar trend can be observed for the Ac-ATAHQD-NH₂ peptide. Figure S5, Supporting Information, shows that the peak appearing at a retention time of 9.2 min, which represents the original peptide, gradually decreases, and a new peak appears at a lower retention time of 9.0 min. After leaving the reaction for 21 h, and recording the HPLC shows another new peak at a retention time of 7.0 min, which represents the formation of the end product of the hydrolytic process.

The chromatograms recorded at pH 11 (Figure 6b) show that there is no hydrolytic process during the period from 0 to 47 min. Only one peak is visible at a retention time of 11.1 min, representing the original peptide fragment. Between 107 and 217 min, a new small peak appears on the HPLC chromatogram at a retention time of 7.9 min. After 21 h, this product peak becomes more intense, indicating that the hydrolytic reaction continues, while the intensity of the original peptide peak decreases as it is consumed during the Ni(II)-induced hydrolytic reaction. In addition, a new small peak appears at a retention time of 5.1 min, indicating the formation of the second product of the hydrolytic reaction. After 7 days, the change in intensity of the peptide and the two different product peaks continues, in accordance with the Ni(II)-induced hydrolytic reaction.

If we compare the chromatograms obtained at the two pH values, we can see that the process proceeds much faster at pH 11. Furthermore, unlike the chromatograms recorded at pH 8, no peak appeared at a retention time of 10.7 min at pH 11 (Figure 6).

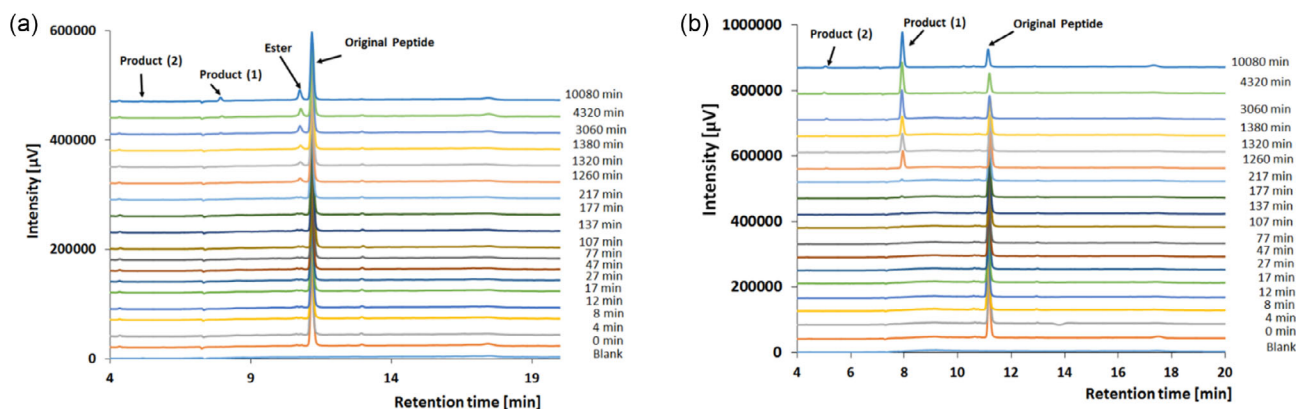


FIGURE 6 | Time-dependent HPLC chromatograms for the Ni(II): tau(385–390) (1:1) ratio system at (a) pH = 8.0 and (b) pH = 11.0 at T = 25°C.

During Ni(II)-induced hydrolysis, an intermediate ester is formed in the first step, followed by hydrolysis of the ester, which cleaves the peptide into two fragments [25]. The process occurring with Ac-KTDHGA-NH₂ is shown in Figure S6, Supporting Information.

For both peptides, the intermediate ester elutes with a lower retention time than the original peptide because the ester has a higher polarity than the original peptide. The difference in retention time between the two products is also due to their different polarities and molecular weights. During the measurement carried out under alkaline conditions, we were unable to detect the ester intermediate. This is obvious since ester hydrolysis is catalyzed by a base, meaning that the formation of the ester intermediate is unstable under strongly basic conditions during the hydrolytic reaction and immediately hydrolysis into the other two products. Integration of the areas under the peaks of the HPLC chromatograms indicates a decrease in peptide concentration, which is accompanied by an increase in the concentration of the two products (Figure S7, Supporting Information).

To clearly demonstrate that the presence of nickel(II) ions is required for the hydrolytic process, the same HPLC measurements were performed in control samples (without nickel(II)). The HPLC measurements showed only the peptide peak at a retention time of 11.1 min, while the peaks of the ester or the other two products were not visible (Figures S8, Supporting Information).

The reaction products were collected and identified by HR-MS measurements in the case of the Ac-KTDHGA-NH₂ peptide (Table 6). During the reaction, the peptide molecule forms an ester in the presence of Ni(II). The molecular formulae of the new ester remains identical to that of the original ligand, as confirmed by the MS values (Table 6). Similarly, the molecular weights of the two end products confirm the hydrolysis process shown in Figure 7. This figure shows the intermediate and final products formed during the hydrolytic steps as they are present in the sample collected from the HPLC, in acidic form (see Experimental Section).

As shown in Figures 7 and S8, Supporting Information, both the intermediate ester and the product contain the NH₂-TXH-sequence, which provides an albumin-like binding site for copper(II) or nickel(II) ions. The complex bound by the (NH₂, N[−], N[−], N_{im}) donor set has an exceptionally high stability, which is also present in the ATCUN motif [16, 42]. Furthermore, since the CD spectra of complexes formed with an albumin-like coordination mode differ from those of (N[−], N[−], N[−], N_{im})-coordinated

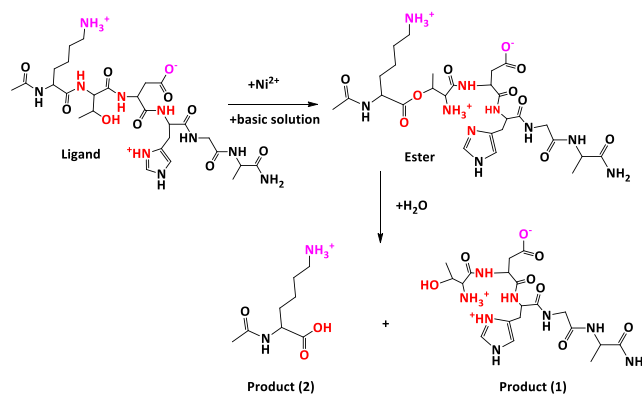


FIGURE 7 | The intermediate and final products formed during the hydrolytic steps of Ac-KTDHGA-NH₂ peptide in acidic form.

complexes, this allows us to monitor the hydrolysis process by CD spectroscopy. Accordingly, the hydrolytic processes occurring in the presence of Ni(II) ions were also followed by CD measurements. We examined the temporal changes in the CD spectra of the Ni(II) complexes of both the tau(385–390) (Ac-KTDHGA-NH₂) and tau(29–34) mutant (Ac-ATAHDQ-NH₂) peptide, in pH 8.0 and 11.0 solutions, over a similar time period as the HPLC measurements. These measurements confirmed the hydrolytic process induced by Ni(II) (Figure 8). There is no visible change in the first 200 min, and the CD spectrum indicates the formation of the Ni(II) complex with (N[−], N[−], N[−], N_{im}) coordination mode. This supports that the hydrolytic reaction is slow at first, even though the pH value is high, and it also means that time is an important factor in this process.

As time progresses, the change observed in the spectra indicates that the hydrolytic process induced by Ni(II) continues, and there is an equilibrium between the complexes with the initial coordination mode and the albumin-type coordination mode, but their ratio is not the same.

After a few days, the measured CD spectra were completely different, indicating albumin-type coordination mode (see Figure 8) and confirming the hydrolytic process. The intensity of the CD spectra is quite low at pH 8.0, as only a part of the Ni(II) is present in the form of a square planar complex, which is necessary for the hydrolytic process. This also explains why the Ni(II)-induced hydrolytic process is slower at this lower pH value. However, CD spectroscopy can only detect the change of the coordination mode as a result of hydrolysis, but no distinction can be made between the intermediate ester and the product, because in both cases the coordination mode of the Ni(II) ion complex is albumin-like (see the scheme in Figure S6, Supporting Information).

TABLE 6 | Retention times and the measured MS data for the products of the hydrolytic reaction, ester, and the original peptide.

Compound	Molecular Formula	Retention Time (min)	Measured Mass (m/z)	Calculated Mass (m/z)
Peptide Ac-KTDHGA-NH ₂	[C ₂₇ H ₄₅ N ₁₀ O ₁₀] ⁺	11.15	669.3335	669.3315
Ester	[C ₂₇ H ₄₅ N ₁₀ O ₁₀] ⁺	10.75	669.3320	669.3315
Product (1) NH ₂ -TDHGA-NH ₂	[C ₁₉ H ₃₁ N ₈ O ₈] ⁺	7.92	499.2278	499.2260
Product (2) Ac-Lys-COOH	[C ₈ H ₁₇ N ₂ O ₃] ⁺	5.09	189.1233	189.1233

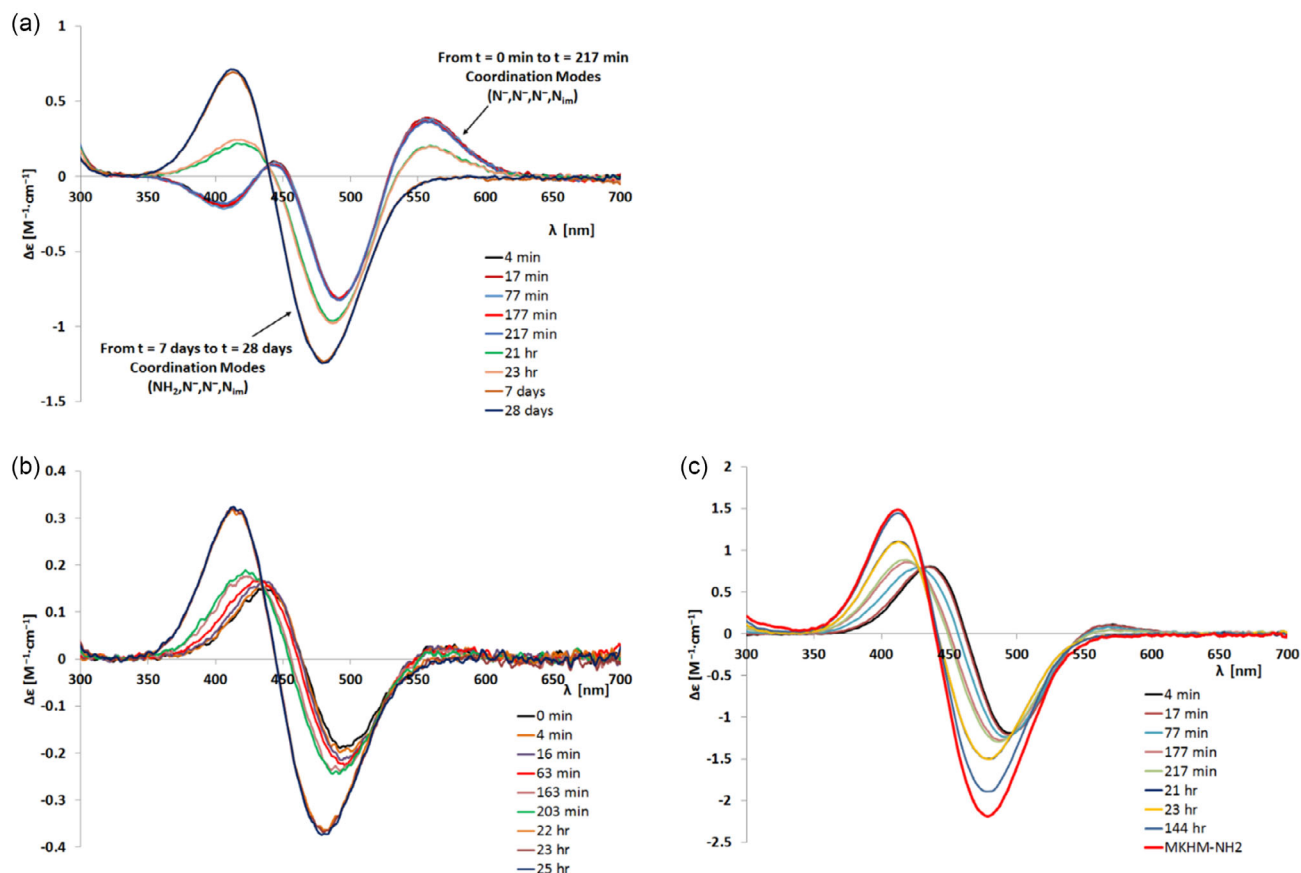


FIGURE 8 | Time-dependent CD spectra for the (a) Ni(II): tau(385–390) (1:1) ratio system at pH = 11.0; (b) Ni(II): Ac-ATAHQD-NH₂ (1:1) ratio system at pH = 8.0 and (c) pH = 11.0; T = 25°C. The CD spectrum of the (NH₂,N[−],N[−],N_{im}) coordinated Ni(II) complex of MKHM-NH₂ peptide is also shown (Figure (c), red curve).

To prove that the position of threonyl or seryl in the peptide sequence relative to the histidyl amino acid is a crucial factor in the hydrolytic process, and that peptides containing the –XTH– part cannot be hydrolyzed in the presence of Ni(II) ions, we also studied the Ni(II) system of the tau(371–376) fragment using CD spectroscopy (Figure S9, Supporting Information). During the measurement of CD spectra lasting nearly 2 days from the start of the reaction, no change was observed in the shape of the CD spectra, which means that the coordination mode of the Ni(II) complex remained the same, the metal ion is bound to the three deprotonated peptide amide and imidazole N donor atoms.

3 | Conclusion

Our previous studies of different tau fragments have shown that the tau(26–33) fragment containing the –TXH– sequence has a significant metal ion binding affinity. These and other previous studies have shown a special role for threonine in the –TXH– sequence.

Accordingly, in our studies we focused on the effect of threonine on complex formation and hydrolytic processes. The Cu(II), Zn(II), and Ni(II) complexes of several protected peptide fragments derived from tau protein containing histidine were studied. In addition to histidine, almost all peptides contain

threonine. The tau(371–376) fragment (Ac-IETHKL-NH₂) containing –XTH– sequence, derived from the R-region of the tau protein showed similar thermodynamic stability in the case of Cu(II), Ni(II), and Zn(II) complexes compared to other one histidine containing peptides, and the threonyl residue adjacent to histidine has no observable effect on the stability and CD and UV-vis spectra.

The two mutants of the tau(29–34) fragment, Ac-ATAHQD-NH₂ and Ac-ATMHQD-NH₂ containing –TXH– sequence showed slightly higher thermodynamic stability in the case of Cu(II), Ni(II), and Zn(II) ion complexes, and resulted in changes in the CD and UV-Vis spectra of the Cu(II) and Ni(II) complexes, similar to the tau(385–390) peptide. This was due to the fact that the threonyl-OH group of the –TXH– moiety axially coordinated to the metal ion. These results supported the previous conclusion, that the threonine has an observable effect only in the peptide containing –TXH– sequence.

Since the hydrolytic process of specific tau protein fragments containing the –TXH– segment in the presence of metal ions may be a crucial factor in the dissociation and accumulation of tau protein in the form of senile plaques, we investigated the Ni(II)-induced hydrolytic process with three tau peptides. CD and HPLC analyses of the tau(385–390) fragment (Ac-KTDHGA-NH₂) and the tau(29–34) mutant peptide (Ac-ATAHQD-NH₂) confirmed Ni(II)-induced hydrolytic processes already at pH 8.

With increasing pH (pH 11.0), the hydrolytic reaction was much faster, indicating the catalytic role of the base in the hydrolysis of the ester during the formation of the two end products. The results of the hydrolytic process were also confirmed by HR-MS measurement.

On the other hand, the hydrolytic process occurring in the presence of metal ions requires threonyl or seryl residues near histidine. Thus, we also investigated the hydrolytic process with the tau(371–376) fragment (Ac-IETHKL-NH₂) derived from the R-region. However, in the case of this peptide fragment, CD measurements showed no hydrolytic activity, which is due to the threonyl position adjacent to the histidine residue.

These results confirm the importance of the presence of the –TXH– moiety in the peptide sequence, not only in terms of the hydrolytic process, but also in terms of stability constants and the spectroscopic parameters of metal complexes.

4 | Experimental Section

4.1 | Materials

All solvents and chemicals used for synthetic purposes were obtained from commercial sources in the highest available purity and used without further purification. All of the N-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (Fmoc-Ala-OH, Fmoc-Thr(tBu)-OH, Fmoc-Met-OH, Fmoc-His(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asp(OtBu)-OH), the Rink Amide resin, and 2-(1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) were purchased from Novabiochem (Switzerland). 2-methyl-2-butanol, N-methyl-pyrrolidone (NMP), 2,2'-(ethylenedioxy)diethanethiol (DOT), N-hydroxybenzotriazole (HOBt), and triisopropyl-silane (TIS) were purchased from Merck, while N,N-diisopropylethylamine (DIPEA) and trifluoroacetic acid (TFA) were Merck Millipore Co. products. Peptide-synthesis grade N,N-dimethylformamide (DMF) and acetic anhydride (Ac₂O) were bought from VWR International, while piperidine, dichloromethane (DCM), diethyl ether (Et₂O), acetic acid (AcOH), and acetonitrile (ACN) from Molar Chemicals Ltd.

All peptides were in the N-acetylated and C-amidated form in order to remove any end-charge effects. The peptides Ac-ATMHQD-NH₂, Ac-ATAHQD-NH₂, and Ac-AAMHQD-NH₂ were synthesized by solid phase peptide synthesizer, while the ligands Ac-IETHKL-NH₂ and Ac-KTDHGA-NH₂ were purchased from SynPeptide Co. Ltd. with purity level above 95%.

Stock solutions (CuCl₂, NiCl₂, ZnCl₂) were prepared from analytical-grade reagents (Reanal Ltd.), and their concentrations were verified gravimetrically via the precipitation of oxinate or complexometric titrations.

4.2 | Peptides Synthesis and Purification

In the case of the synthesized ligands solid phase peptide synthesis was performed with the aid of a microwave-assisted Liberty Peptide Synthesizer (CEM, Matthews, NC). Fmoc/tBut method

and TBTU/HOBt/DIPEA activation strategy were used. Cleaving of α-amino protecting group of amino acids and resin was performed with a 20% V/V piperidine and 0.1 M HOBt in DMF solution at 75°C with 30 W microwave power for 180 s. Excess of amino acids and 30 W microwave power for 300 s were used for coupling at 75°C in the presence of 0.5 M HOBt and 0.5 M TBTU in DMF as an activator and 2 M DIPEA in NMP as an activator base. The free amino terminus was treated with DMF containing 5% V/V Ac₂O and 6% V/V DIPEA to obtain the acetylated amino group. After the completion of the synthesis, the peptides were cleaved from the resin with the simultaneous removal of the side chain protective groups, by treatment with a mixture of TFA/TIS/H₂O/DODT in 94/2.5/2.5/1% V/V for 2 h at room temperature. The solution containing the synthesized peptide was separated from the resin by filtration. Cold Et₂O was used to precipitate the crude peptide from the solution and to wash from the contaminants of the chemical reagents of the synthesis and cleaving agents. After the filtration process, the product was dried, dissolved in water, purified, and lyophilized.

The purification of the peptides was carried out by preparative RP-HPLC using a Preparative scale-up LC-20A HPLC-DAD system (Shimadzu) chromatography system with detection at 222 nm. The column used for the purification was a Synergi, Fusion-RP C18 250 × 10 mm (80 Å pore size, 4 μm particle size). The peptides were eluted at the flow rate of 3 mL/min, where the eluent was the mixture of solvent A (H₂O containing 0.1% TFA) and B (ACN containing 0.1% TFA) in different percentage depending on the pertinent method.

The purity of the synthesized ligands was checked by analytical reversed-phase high performance liquid chromatography (RP-HPLC) and electrospray ionization-mass spectrometry (ESI-MS) analysis, and it was greater than 90%. The concentration of the ligands were checked by pH-potentiometric measurements.

4.3 | Potentiometric Measurements

pH-potentiometric titrations were completed on 3.00 mL samples. The concentration of the ligands was in the range from 1 to 2 mM, and their accurate concentration was determined by using carbonate free stock solution of potassium hydroxide of known concentration ≈ 0.2 M. The selected ratios for M:L were 1:1, 1:2, and 1:3 for the titration, Argon gas was being bubbled above the surface of the solution to ensure the absence of CO₂ gases. The samples were being stirred by a VELP scientific magnetic stirrer.

All pH-potentiometric measurements were carried out at 298 K and at constant ionic strength of 0.2 M KCl. The pH measurements were made using a MOLSPIN pH-meter equipped with a 6.0234.100 and 6.0234.110 combination glass electrode (Metrohm) and the titrant dispensed by means of a computer controlled MOL-ACS buret. During the titrations the following equilibrium conditions were used: the system was in equilibrium if the maximum change of pH is 0.001 unit for 30 s or if the maximum change of potential is 0.03 mV for 9 s. The recorded pH readings were converted into hydrogen ion concentration as described by Irving et al. [43]. Protonation constants of the

ligands and overall stability ($\log\beta_{\text{pqr}}$) constants of the metal complexes were calculated by means of general computational programs (PSEQUAD [44] and SUPERQUAD [45]) based on the Equations (1) and (2).

$$pM + qH + rL \rightleftharpoons M_p H_q L_r \quad (1)$$

$$\beta_{\text{pqr}} = [M_p H_q L_r] / [M]^p [H]^q [L]^r \quad (2)$$

Some derived constants were calculated according to Equations (3) and (4).

$$\log K(M - N_{\text{im}}) = \log \beta(MH_X L) - \log \beta(H_X L), (X=0 \text{ or } 1) \quad (3)$$

$$pK_{\text{amide}} = (\log \beta(MH_X L) - \log \beta(MH_{X-n} L)) / n \quad (n=1, 2 \text{ or } 3) \quad (4)$$

The concentration distribution curves were generated by the MEDUSA program [46] using the protonation constants of the ligands and the stability constants of the metal ion complexes.

4.4 | Spectroscopic Measurements

UV-Vis Spectroscopy: The same concentration range used for the pH-potentiometry was selected to register the UV-Vis spectra of the copper(II) and nickel(II) complexes. The measurements were carried out using a VWR UV1600 PC scanning spectrophotometer at a wavelength range of 200–900 nm.

CD Spectroscopy: A JASCO J-810 spectropolarimeter was used to perform the CD measurements. CD spectra of the copper(II) and nickel(II) complexes were acquired from 220 to 800 nm using 1 cm and 1 mm cells and at the same concentration as applied for pH-potentiometric measurements.

Electrospray Ionization-Mass Spectrometry: The Bruker maXis II QTOF ESI-TOF instrument was used to perform the ESI-TOF-MS analyses in the positive mode for the synthesized ligands with the concentration of 0.1 μM and also for the products of the hydrolytic process.

4.5 | Sample Preparation for the Hydrolysis Studies

In all cases, the ratio was equimolar (1:1), with a slight excess of the peptide to eliminate the formation of nickel(II) hydroxide precipitate. The peptides were dissolved in 20 mM HEPES buffer to achieve a concentration of ≈ 1 mM, which was then adjusted to pH 8.0 and 11.0 with 0.2 M KOH and HCl. After pH adjustments the samples were incubated at 25°C. Finally, the metal ion was added (in the case of the control samples, the same volume of distilled water was added instead), marking the $t=0$ point. The reaction vessels were hermetically sealed to prevent the evaporation of water and were only opened for the purpose of taking samples of 50 μL . The reaction was halted by the addition of 200 μL , 1% solution of HPLC-grade trifluoroacetic acid, after which the samples were stored in a refrigerator at $\approx 7^\circ\text{C}$ until the analytical HPLC and MS measurement was conducted.

The final concentration of the protein fragment (L) at the $t=0$ point, following the halting of the reaction, was ≈ 0.17 mM.

4.6 | Reverse-Phase High Performance Liquid Chromatography Measurements

The application of the RP-HPLC technique was important not only to prove the purity of the synthesized peptides, but also to prove the presence of the hypothesized metal ion-induced hydrolytic process.

For the purification of the protein fragment (L), a semipreparative and an analytical Jasco instrument were used, monitoring the wavelength at 222 nm. A gradient elution with 0%–30%–0% of solvent B was carried out using solvent A (0.1 V/V% TFA in Milli-Q water) and solvent B (0.1 V/V% TFA in acetonitrile) at a flow rate of 1.00 mL/min. A Teknokroma EUROPA PROTEIN C18 chromatographic column (250 $\times$ 4.6 mm, 300 Å pore size, 5 μm particle size) was used for analytical measurements. A flow rate of 3 mL/min and two semipreparative columns (EUROPA PROTEIN C18 chromatographic column (250 $\times$ 10 mm, 300 Å pore size, 5 μm particle size), EUROPA PROTEIN C8 chromatographic column (250 $\times$ 10 mm, 300 Å pore size, 5 μm particle size)) in linear succession are used to separate and collect the synthesized product with the instrument.

For the hydrolysis studies, the measurements were performed on a Waters Alliance 2995 separations module equipped with a Waters Alliance Series Column Heater/Cooler, a Waters 2996 PDA detector, and a Waters 2487 Dual λ Absorbance Detector. Chromatographic data acquisition and handling were performed using EmpowerTM 3 CDS (Waters, Milford, MA, USA). Separation of the components was achieved on an XBridge BEH Shield RP18 (130 Å, 2.5 μm , 4.6 mm $\times$ 75 mm) column equipped with an ACQUITY Column In-Line Filter (Waters, Milford, MA, USA). A gradient method was used for the separation with 0.1% TFA in water as solvent A and 0.1% TFA in acetonitrile as solvent B. The flow rate of the mobile phase was 1.00 mL/min, and the column temperature was set to 25°C. Injection volume was 10.0 μL , and the detection wavelength on the UV detector was 222 nm.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Fig. S1:** UV-Vis spectra of Cu(II) complexes of Ac-IETHKL-NH₂ (c(L) = c(M) = 1.6 mM) (a) and Ac-ATMHQD-NH₂ (c(L) = c(M) = 1.0 mM) (b) in function of pH. **Supporting Fig. S2:** CD spectra of Cu(II) complexes of Ac-IETHKL-NH₂ (a), Ac-AAMHQD-NH₂ (b), Ac-ATMHQD-NH₂ (c), and Ac-ATAHQD-NH₂ (d) at copper(II)-ligand 1:1 ratio in function of pH. **Supporting Fig. S3:** UV-Vis spectra of Ni(II) complexes of Ac-IETHKL-NH₂ (c(L) = c(M) = 1.6 mM) (a) and Ac-AAMHQD-NH₂ (c(L) = c(M) = 2.0 mM) (b). **Supporting Fig. S4:** CD spectra of Ni(II) complexes of Ac-IETHKL-NH₂ (1:1) (a); Ac-AAMHQD-NH₂ (1:1) (b), and Ac-ATMHQD-NH₂ Ligand (1:1) (c). **Supporting Fig. S5:** Time-dependent HPLC chromatograms for the Ni(II): Ac-ATAHQD-NH₂ (1:1) ratio system at pH = 8.0 (T=25°C). **Supporting Fig. S6:** Mechanism of Ni(II)-ion-induced hydrolysis [25] of Ac-KTDHGA-NH₂ peptide. **Supporting Fig. S7:** Relation between area of peak in the HPLC chromatograms with time for Ni(II): tau(385-390) (1:1) ratio system at pH = 8.0 (a); pH = 11.0 (b) and for Ni(II): Ac-ATAHQD-NH₂ (1:1) ratio system at pH = 8.0 (c) (T = 25°C). **Supporting Fig. S8:** Time-dependent HPLC chromatograms for tau(385-390) peptide at pH = 8.0 (a) and pH = 11.0 (b) (T = 25°C). **Supporting Fig. S9:** Time-dependent CD Spectra for the Ni(II): tau(371-376) 1:1 ratio system at pH = 11.0 (T = 25°C). **Supporting Table S1:** CD spectra (λ_{max} [nm] and $\Delta\epsilon$ [M⁻¹·cm⁻¹]) values for Cu(II) complexes (T = 298 K, I = 0.2 M KCl).