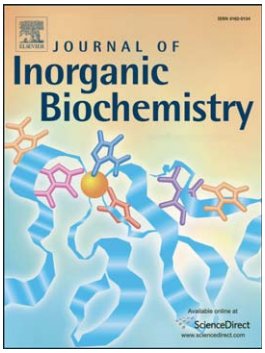

Copper(II), nickel(II) and zinc(II) complexes of the N-terminal nonapeptide fragment of amyloid-β and its derivatives

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Copper(II), nickel(II) and zinc(II) complexes of the N-terminal nonapeptide fragment of amyloid-β and its derivatives

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Abstract

Copper(II), nickel(II) and zinc(II) complexes of the nonapeptide fragment of amyloid-β Aβ(1-9) (NH_2-DAEFRHDSG-NH_2) and its two derivatives: NH_2-DAAAAHAAA-NH_2 and NH_2-DAAAAAHAA-NH_2 have been studied by potentiometric, UV-visible and CD spectroscopic methods. The results reveal the primary role of the amino terminus of peptides in copper(II) and nickel(II) binding. The formation of dinuclear complexes was also possible in the copper(II) containing systems but only the first six amino acids from the amino terminus were involved in metal binding in the physiologically relevant pH range. The coordination chemistry of the two alanine mutated peptides is almost the same as that of the native nonapeptide, but the thermodynamic stability of the copper(II) complexes of the mutants is significantly reduced. This difference probably comes from the secondary interactions of the polar side chains of Asp, Glu, Ser and Arg residues present in the native peptide. Moreover, this difference reveals that the amino acid sequence of the N-terminal domains of amyloid peptides is especially well suited for the complexation with copper(II) ions.

Keywords:

copper(II), nickel(II), zinc(II), amyloid-β, stability constants, CD spectroscopy

1. Introduction

The role of metal ions in the biological processes associated with Alzheimer's disease gave a big impetus to the studies on the coordination chemistry of amyloid-β peptide and its fragments. Huge number of papers has been published in this field in the past few years including several reviews [1-12]. Most of the previous studies were devoted to the characterization of the copper(II) and zinc(II) complexes but the metal ion promoted redox chemistry of the peptides is also widely investigated. In spite of the extremely high number of related publications the metal binding sites and the data reported for the thermodynamic stability of complexes remain contradictory. This dichotomy partly comes from the low solubility of the 42-residue amyloid-β peptide and especially its metal complexes. At the same time it is widely accepted that the N-terminal domain of amyloid-β is the primary metal binding site which is rich in polar and coordinating side chains. It is also obvious from the previous studies that the N-terminal hexadecapeptide fragment, amyloid-β(1-16), is the most promising candidate for stable interactions with transition metal ions. Its sequence corresponds to NH_2-DAEFRHDSGYEVHHQK-NH_2 containing a free amino terminus from Asp(1), three imidazole-N donors from His(6), His(13) and His(14) and four carboxylates from Asp(1), Glu(3), Asp(7) and Glu(11) residues. Moreover, the peptide contains a phenolate-O and amino-N donors from Tyr(10) and Lys(16) residues, respectively, but they are generally not considered as metal binding sites. The high number of protonation sites and the solubility problems make even the acid-base characterization of the peptide difficult. As a consequence, the description of metal binding under physiological conditions only was in the focus of most publications. The data obtained under these conditions are relevant from a biological point of view but do not provide a complete description of the metal-ligand interaction.

The first pH-dependent solution equilibrium studies on the copper(II) complexes of various fragments of amyloid-β were published more than ten years ago, but only the species formed in equimolar samples were taken into account in these reports [13-14]. The results obtained in our laboratories in the last few years [15-18], however, provided a more comprehensive picture on the complex formation with these peptides. The synthesis of the PEG (polyethyleneglycol)-conjugate of the peptide Aβ(1-16)PEG helped to overcome the solubility problems, while the use of short fragments Aβ(1-6) and Ac-Aβ(8-16)Y10A made it possible to suggest the major metal binding sites in solution. It was found that the hexadecapeptide can bind as much as 4 copper(II) ions and the terminal-NH_2, His(6), His(13) and His(14) residues were identified as the major copper(II) binding sites [15]. Furthermore, a high zinc(II) binding affinity of the peptide was reported with a preference for the internal histidyl sites in metal binding [16]. In the case of nickel(II) the formation of only mono- and di-nuclear species was suggested via the terminal amino and internal histidyl sites [17]. Moreover, it was reported that zinc(II) ions cannot replace but can alter the distributions of copper(II) among the available binding sites [18]. More recent studies provided further support for this observation [19,20].

The solution equilibria of the copper(II), nickel(II) and zinc(II) complexes of amyloid fragments have been described in our previous publications [15-18] but some structural details of these interactions remained unanswered, yet. For example, a high number of different coordination isomers of the mononuclear species can coexist in these systems. Among them the binding mode of His(6) residue is probably one of the most questionable, because there are only five amide groups between Asp(1) and His(6) sites. Thus, the saturation of the coordination spheres of two copper ions is not possible towards the N-termini providing a chance for the similar processes at the C-terminal side of the peptide. Systematic equilibrium and structural studies on the complexes of additional small fragments

can help to answer this question. Now in this paper we report the synthesis of the nonapeptide fragment Aβ(1-9) (=NH_2-DAEFRHDSG-NH_2) of the native peptide and its two derivatives: NH_2-DAAAAHAAA-NH_2 and NH_2-DAAAAAHAA-NH_2. The comparison of the data obtained for the complexes of the native and alanine mutated fragments helps to understand the influence of weakly coordinating side chains in Aβ(1-9). The positions of histidines are different (H6 and H7) in the two alanine-mutated fragments providing a chance for the distinction of amide deprotonation and metal binding of amide nitrogens at the N-or C-terminal side of the histidyl residues.

2. Experimental

2.1. Peptide synthesis and other materials

The N-terminally free peptides were obtained by solid phase peptide synthesis using the Fmoc technique with the sequences of NH_2-AspAlaGluPheArgHisAspSerGly-NH_2 (DAEFRHDSG), NH_2-AspAlaAlaAlaAlaHisAlaAlaAla-NH_2 (DAAAAHAAA) and NH_2-AspAlaAlaAlaAlaAlaHisAlaAla-NH_2 (DAAAAAHAA). All chemicals and solvents used for synthesis were obtained from commercial sources in the highest available purity and used without further purification. Rink Amide AM resin, 2-(1-H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and all N-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (Fmoc-Asp(OtBu)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Trt)-OH, Fmoc-Ser(tBu)-OH and Fmoc-Gly-OH) were from Novabiochem (Switzerland). N,N-diisopropyl-ethylamine (DIEA), trifluoroacetic acid (TFA) and analytical grade N,N-dimethylformamide (DMF), were purchased from Merck Kft. N-methyl-pyrrolidone (NMP), 1-hydroxybenzotriazole hydrate (HOBt·H_2O), 2,2'-

(ethylenedioxy)diethanethiol, triisopropylsilane (TIS), 2-methyl-2-butanol and HPLC grade trifluoroacetic acid were Sigma-Aldrich products. Dichloromethane (DCM), diethyl ether (Et_2O), acetic acid (AcOH) and piperidine were Molar solvents as well as acetic anhydride and HPLC grade acetonitrile (ACN) were from VWR.

All peptides were synthesized by solid phase peptide synthesis using a microwave-assisted Liberty 1 Peptide Synthesizer (CEM, Matthews, NC), introducing the amino acid derivatives following the TBTU/HOBt/DIEA activation strategy on the Rink Amide AM resin. Removal of the Fmoc was carried out by means of 20% piperidine/0.1 M HOBt·H_2O in DMF at 75 °C with 35 Watts microwave power for 180 s. 0.5 M HOBt·H_2O /0.5 M TBTU in DMF and 2 M DIEA in NMP were used for coupling at 75 °C with 25 Watts microwave power, for 300 s, adding 4 times excess of amino acids. Finally, the N-terminal Fmoc group was removed as described before. Cleaving of the peptides from their respective resins and the simultaneous removal of the side chain protective groups were carried out by treatment with a mixture containing TFA/TIS/H_2O/2,2'-(ethylenedioxy)diethanethiol (94/2.5/2.5/1 v/v) at room temperature for 1.5 h. After cleaving each solution the free peptide was separated from the resin by filtration. Cold diethyl ether was used to precipitate the crude peptides from the pertinent solution and to wash from the contaminants of the reagents of the synthesis and cleaving agents. After separating from it, the products were dried under argon gas, redissolved in water, and finally lyophilized. The purity of the peptides was checked by analytical rp-HPLC analyses using a Jasco instrument, equipped with a Jasco MD-2010 plus multiwavelength detector. The analyses were performed on a Vydac C18 chromatographic column (250 x 4.6 mm, 300 Å pore size, 5 μm particle size) by eluting 2% of solvent A (0.1% TFA in acetonitrile) and 98% of solvent B (0.1% TFA in water) at a flow rate of 1 mL/min monitoring the absorbance at 222 nm. Analytical rp-HPLC for all peptides and pH-dependent ^1H NMR spectra for the Aβ(1-9) fragment was used to check the purity of the

peptides. Potentiometric titrations also confirmed the purity and the identity of the peptides. The purity was greater than 95% for all three peptides.

$CuCl_2$, $NiCl_2$ and $ZnCl_2$ containing metal ion stock solutions were prepared from analytical grade reagents and their concentrations were checked by gravimetry via the precipitation of oxinates.

2.2. Potentiometric measurements

The pH-potentiometric titrations were performed with the use of carbonate-free stock solution (0.2 M) of potassium hydroxide in 3 mL samples at 2 mM ligand concentration. The metal ion to ligand ratios were selected as 1:1 and 2:1 for binary systems. While the equilibration of the nickel(II)-peptide systems required a few minutes for each titration point in the pH range 8.0-9.0 the copper(II) and zinc(II) complexes were formed in fast reactions. This is reflected in the increased standard deviations of stability constants of the nickel(II) complexes comparing with those of the other two metal ions. To ensure the absence of oxygen and carbon dioxide, argon was bubbled through the samples and they were stirred by a VELP Scientific magnetic stirrer. All pH-potentiometric measurements were carried out at a constant ionic strength of 0.2 M KCl at 298 K. A MOLSPIN pH-meter equipped with a 6.0234.100 combination glass electrode (Metrohm) and a MOL-ACS microburette controlled by a computer was used to carry out pH measurements. The recorded pH values were converted to hydrogen ion concentration. Protonation constants of the ligands and overall stability constants ($\log \beta_{pqr}$) of the metal complexes were calculated by means of the general computational programs, (PSEQUAD and SUPERQUAD) as described in our previous publications [17,21]. The equilibrium constants were defined by equations (1) and (2):

$$pM + qH + rL = M_pH_qL_r \quad (1)$$

$$\beta_{pqr} = \frac{[M_pH_qL_r]}{[M]^p \cdot [H]^q \cdot [L]^r} \quad (2)$$

2.3. Spectroscopic measurements

A Perkin Elmer Lambda 25 scanning spectrophotometer was used to record the UV-visible (UV-Vis) spectra of the copper(II) and nickel(II) complexes in the wavelength range of 250 to 800 nm. The ligand and metal ion concentrations of the samples were the same as reported for the pH-potentiometric measurements.

CD spectra of the same complexes were recorded on a JASCO J-810 spectropolarimeter in the 220-800 nm wavelength range using 1 and/or 10 mm cells at the same concentration as used for pH-potentiometry.

ESI-TOF-MS analysis was carried out with a Bruker micrOTOF-Q 9 ESI-TOF instrument in the negative mode. The measurements were performed in water at $c_L=3.5\times10^{-4}$ M at Cu(II): L = 1.8:1 ratio at pH=7 by adding 0.2 M KOH solution. Temperature of drying gas (N_2) was 180 °C and the pressure of the nebulizing gas (N_2) was 0.3 bar. The capillary voltage applied was 4000 V and the spectra were accumulated and recorded by a digitalizer at a sampling rate of 2 GHz.

3. Results and Discussion

Potentiometric titrations were used to determine the protonation constants of the ligands and these values are included in Table 1. The data reported for the hexapeptide Aβ(1-6) are also included for comparison [15]. It is clear form Table 1 that the number of protonation sites of the three ligands is different. The terminal Asp and His(6) or His(7) residues are present in all molecules and their amino- and imidazole-N donors represent the most basic sites. A slight overlap can be in their protonation reactions but the highest pK values describe well the deprotonation of the terminal ammonium group. Moreover, these values are in a good agreement with the pK values of other terminally free histidine containing peptides [22]. The other pK values belong to the carboxylate functions of aspartyl and glutamyl residues, the γ-carboxylic group of glutamic acid being generally the less acidic site among them [23]. Although the metal binding properties of the three peptides are quite similar, the speciation for the three metal ions is different and the results are discussed separately for each metal ion.

Table 1

3.1. Copper(II) complexes

The stability constants of the copper(II) complexes were determined by pH-metric titrations in both equimolar samples and in the presence of excess metal ion or ligand. The data are listed in Table 2 and the stepwise pK values of various protonated complexes are also shown. The computer evaluation of the experimental data unambiguously ruled out the formation of bis(ligand) complexes in measurable concentration but dinuclear species were formed in all systems. Table 2 reveals that the speciation of the four systems is rather similar and it is depicted only for the complexes of the native nonapeptide in Figure 1.

Table 2

Figure 1

The equilibrium data and structural characterization of the complexes of the C-terminally shortened hexapeptide have already been published in our previous paper [15] but the data are also included in Table 2 for comparison. It can be seen form Table 2 that the complex formation processes of the native hexa- and nona-peptides are quite similar suggesting the existence of the same coordination modes for all species with the same stoichiometry. The pH-dependent concentration distribution curves in Figure 1.a. reveal the consecutive deprotonation of the various protonated complexes. It is a common feature of the copper(II) complexes of simple oligopeptides when the terminal amino group is the primary metal binding site [24-26]. The results of UV-Vis and CD spectroscopic measurements provided further evidence for the coexistence of these coordination modes in slightly acidic samples. The spectroscopic parameters calculated for the mononuclear copper(II) species are included in Table 3.

Table 3.

It can be seen from the comparison of Table 3 and Figure 1 that the absorption maximum of the species [CuL] is recorded at 696 nm but measurable CD activity cannot be obtained below pH 5.5. This observation rules out the binding of amide nitrogens in this species. On the other hand, the stability constants of [CuL] are much higher than those of the (amino, carbonyl) or (amino, carboxylate)-coordinated common peptide complexes [22,26]. These data suggest the binding of amino and carboxylate functions of the terminal aspartyl residue in the formation of a 6-membered chelate, which is supported by a macrochelate from the distant histidyl residue (see Scheme 1.a). Similar stability enhancement has already been reported for peptides containing distant histidyl residues [21,22]. The protonated complexes ([CuH_2L] and [CuHL]) are present in low concentration and formed in overlapping processes. Their

absorption maxima cannot be accurately determined but occurs above or around 700 nm suggesting the same coordination mode of the amino terminus with protonated histidyl and glutamyl side chains.

Subsequent deprotonation reactions or the formation of species [$CuH_{-n}L$] (n=1-3) is accompanied with a significant blue shift of the absorption maxima and the appearance of new CD extrema by increasing intensity (see data in Table 3). Similar observations have already been reported for the corresponding hexapeptide fragment of amyloid-β and were explained by the copper(II) coordination of subsequent amide nitrogens starting from the amino terminus [15]. These data strongly support that terminal amino and subsequent amide nitrogens are the primary metal binding sites up to pH 9.0. However, a new CD extrema can be recorded in more alkaline samples with a low intensity positive Cotton effect around 660 nm. The species [$CuH_{-3}L$] predominates in these alkaline samples and its CD spectrum is plotted with those of the same species of Aβ(1-4) and Ac-Aβ(1-6) [15] and the dinuclear complex [$Cu_2H_{-6}L$] of Aβ(1-9) in Figure 2. The comparison of the CD spectra of the mononuclear complex of Aβ(1-9) with those of Aβ(1-4) and Ac-Aβ(1-6) reveals the co-existence of coordination isomers in the species [$CuH_{-3}L$]. Both isomers are 4N-coordinated complexes but the primary coordinating donors start from the amino terminus or from the internal histidyl sites (see Scheme 1.b and c). The ratio of the (NH_2,N^-,N^-,N^-) and (N^-,N^-,N^-,N_{im},) isomers can be estimated from the intensity of CD spectra and the value 3:1 was obtained reflecting the predominance for binding to the amino group.

Scheme 1

Figure 2

Formation of dinuclear complexes with the general stoichiometry of [$Cu_2H_{-n}L$] (n=3-5 or 6) was also recorded with all peptides. It can be seen from Figure 1.b that the formation of these species starts above pH 6 in parallel with the coordination of amide nitrogens. ESI-MS

measurements provided a direct evidence for the existence of dinuclear species. Figure 3 is used to demonstrate the mass spectra of the 2:1 copper(II)-Aβ(1-9) samples in the physiological pH range. In accordance with the speciation curves (see Figure 1.b.) the existence of two major species the mononuclear $[CuH_{-1}L]^{2-}$ and the dinuclear $[Cu_2H_{-3}L]^{2-}$ can be unambiguously seen in the mass spectra. The agreement of the isotope distribution of the calculated and experimental spectra justifies the coexistence of the mono- and di-nuclear complexes under these conditions.

Figure 3

In the case of the hexapeptide Aβ(1-6) the binding of the second copper(II) ion can occur only at the N-terminal side of the histidyl residue and only two amide nitrogens are available for copper(II) binding in agreement with the $[Cu_2H_{-5}L]$ stoichiometry of the dinuclear complex at high pH. For the nonapeptide, the existence of the species $[Cu_2H_{-6}L]$, can also be calculated. The corresponding pK value is, however, very high (pK =10.66). The high pK value cannot rule out the formation of a hydroxo complex but from the CD spectra the existence of a new coordination mode can also be suggested. In the case of the nonapeptide Aβ(1-9) there are three additional amino acids on C-terminal side of histidine providing a chance for the (N_{im},N^-,N^-,N^-) coordination mode towards the C-termini. The chelate ring sizes are (7,5,5) for this binding mode (Scheme 1.d.) while it is (6,5,5) towards the N-termini (Scheme 1.c.). It is evident that the binding mode with 7-membered chelate has reduced stability but the coexistence of the two coordination modes is possible at high pH and this is in accordance with the relatively low intensity of the positive Cotton effect in the CD spectra of the dinuclear samples at high pH values (see Figure 2.d).

It is clear from Table 2 that the stoichiometry of major species formed with the alanine mutated nonapeptides is very similar to that of Aβ(1-9). The stability constants especially for the dinuclear species are, however, significantly different. This can be demonstrated by Figure

4 where the distribution of copper(II) among the three peptide ligands is plotted as a function of pH. It can be seen from this Figure that the alanine mutated peptides are less effective metal binders than the native peptide fragments. The side chain carboxylate functions of Glu(3) and Asp(7) are missing from the mutants and this results in a big change in the overall charge of the peptides. The fully deprotonated form of Aβ(1-9) is L^{3-}, while it is only L^{-} for NH_2-DAAAAHAAA-NH_2 and NH_2-DAAAAAHAA-NH_2. This change in the charge of the ligands will influence the overall charge of the corresponding complexes, too. Namely, the most common dinuclear species [$Cu_2H_{-5}L$] is a two-, three- or four-negatively charged species for the mutants, and native hexa- and nona-peptides, respectively. However, it is also clear from Table 2 that the different charges of the complexes cannot give the sufficient explanation for the differences in their stability constants. The stability order of the peptide complexes is: Aβ(1-6) > Aβ(1-9) >> mutants. This trend suggests some weak axial interactions form the side chain carboxylate residues. Moreover, the presence of polar side chains in the native fragments may also have some contribution to the overall stability of the copper(II) complexes of amyloid fragments. Similar observations have been reported recently by Alies et al. for the copper(II) complexes of N-terminally modified peptides [27]. In general, the stabilizing role of weakly coordinating or polar side chains have already been demonstrated in reviews, by several authors [24-26,28,29]. These weak interactions, however, do not change the major structural properties of the various complexes as it can be seen from the comparison of spectroscopic parameters in Table 3. The binding modes in the mononuclear copper(II) complexes of alanine mutants are the same as those of Aβ(1-9) and even their stability is not much reduced. The high decrease of stability is obtained for the dinuclear species of the mutant peptides and it is reflected in the absence of [$Cu_2H_{-6}L$] species of NH_2-DAAAAHAAA-NH_2 containing only five amide groups between the anchoring amino and histidyl sites. The pK value for the formation of this deprotonated species is,

however, very high even for the native fragment (pK = 10.66) and it is further increased for the other mutant with six amides between the anchoring sites (pK = 11.76).

Figure 4

Another small structural difference can be seen in the high pH CD spectra of the peptides. The development of the positive Cotton effect at 660 nm in the [$CuH_{-3}L$] species of Aβ(1-6) and Aβ(1-9) was interpreted by the coexistence of coordination isomers with a preference for the binding to the amino terminus. The intensity of these CD extrema is especially low for the mutants reflecting a reduced tendency for binding at the internal histidyl sites in accordance with the reduced affinity for the formation of dinuclear species.

3.2. Nickel(II) complexes

Nickel(II) is not considered as an essential element for humans and it is not involved in the development of neurodegeneration. At the same time, nickel(II) ions play an important role in the biochemical processes of various plants and microorganisms. His and/or Cys residues are the most common nickel(II) binding sites in the corresponding proteins justifying the studies on nickel(II) peptide complexes. Stability constants of the nickel(II) complexes are included in Table 4, while the spectroscopic data for the major nickel(II) species were listed in Table 3. The absence of dinuclear complexes in the nickel(II) containing systems represent the major difference in the complex formation processes of copper(II) and nickel(II) ions. The titration of 2:1 = nickel(II):peptide samples resulted in precipitation above pH 8.0 in all cases and it was not dissolved completely even by pH 12.0. This suggests that dinuclear species can exist only under extremely alkaline conditions without any biological significance. The stoichiometry of the mononuclear complexes is, however, very similar to that of copper(II). The formation of 3 or 4 species was obtained from the computer calculations but only 2 of

them ([NiL] and [$NiH_{-3}L$]) are present in measurable concentration. The species [NiL] exists between pH 6 to 8 but without measurable CD activity. The weak absorption bands clearly indicate that nickel(II) ion has an octahedral coordination geometry in this species and the same binding mode as reported for the corresponding copper(II) species can be suggested (see Scheme 1.a.). The characteristic yellow colour of the samples above pH 8.0 indicates the formation of square planar complexes in this pH range. Only the intensity of both UV-visible and CD spectra is changing by increasing pH supporting the cooperative deprotonation of three amide nitrogens and the formation of [$NiH_{-3}L$] in alkaline samples. In principle, the coordination isomers (Scheme 1.b and 1.c) can be present in the nickel(II) containing systems, too. The comparison of the CD spectra of the [$NiH_{-3}L$] species of Aβ(1-9) with those of Aβ(1-4) and Ac-Aβ(1-6), however, unambiguously supports the exclusive binding of nickel(II) to the amino terminus of the nonapeptides (Figure 5). Moreover, the comparison of the stability constants of the three nonapeptide complexes rules out any stabilizing or destabilizing effect from the non-coordinated side chain residues of Aβ(1-9). This observation is not surprising if one takes into account the strictly square planar geometry of these nickel(II) complexes. Furthermore, the involvement of polar side chains were suggested to be more effective in the dinuclear copper(II) complexes but this type of species is not formed with nickel(II).

Table 4

Figure 5

3.3. Zinc(II) complexes

Stability constants of zinc(II) complexes were also determined by potentiometric titrations and these data are included in Table 5. Before the interpretation of the results it is important to note that the amino terminus of Aβ(1-6) was suggested as the primary copper(II) and

nickel(II) binding site, while the internal histidines (especially His13 and His14) were suggested for zinc(II) [12]. On the basis of these results, relatively low zinc(II) binding affinity can be expected for the nonapeptides. The experimental data are in agreement with this expectation. The speciation of the three nonapeptides is very similar and precipitation of zinc(II)-hydroxide prevent the measurements in alkaline samples. [ZnL] is the major species in all cases and its coordination geometry support the formation of β-alanine like 6-membered chelate supported by the macrochelation from the side chain imidazole. This binding mode enhances the stability constants as compared to simple oligoglycines but does not prevent hydrolysis above pH 8. The species $[ZnH_{-1}L]$ is probably a mixed hydroxo complex and the lowest value is obtained for the native nonapeptide. In other words it means that the uncoordinated polar side chains of Aβ(1-9) suppress the hydrolytic reactions and in an indirect way they contribute to the enhanced zinc(II) binding of amyloid peptides.

Table 5

4. Conclusions

Previous studies on the copper(II), nickel(II) and zinc(II) complexes of amyloid peptides [15-17] have already demonstrated the outstanding copper(II) and nickel(II) binding affinity of the amino terminal domain of the peptide, some structural details of the interactions, however, remained unanswered. The results of this study provide a significant contribution to the better understanding of these questions. The combined application of potentiometric and UV-visible, CD and mass spectrometric measurements on the complexes of the N-terminal nonapeptide fragment, Aβ(1-9), undoubtedly prove the primary role of the amino terminus in copper(II) and nickel(II) binding. The macrochelate created by the histidine nitrogens,

however, enhances the thermodynamic stability of the species existing in the physiological pH range ([CuL] and [$CuH_{-1}L$]). In the case of copper(II) the formation of dinuclear complexes was also recorded with the involvement of His(6) residue in copper(II) binding. The comparison of the present data with those reported earlier for Aβ(1-6) made it possible to clarify the binding modes around the histidyl site of the peptide. It was found that only the first six amino acids from the amino terminus are involved in metal binding in the physiological pH range. Among them, the amino and the subsequent amide nitrogens are the primary ligating sites but the coexistence of two different 4N complexes (NH_2,N^-,N^-,N^-) and (N^-,N^-,N^-,N_{im}) is also possible in alkaline samples with a high preference for the former isomer. Moreover, the deprotonation of amide nitrogens towards the C-terminus cannot be excluded but only in the dinuclear species [$Cu_2H_{-6}L$] formed above pH 11.0.

Another important conclusion can be obtained from the comparison of the data reported for Aβ(1-9) and its alanine mutated counterparts. The major metal binding sites of the two mutants are the same as those of the native nonapeptide, but the thermodynamic stability of the copper(II) complexes of the mutants is significantly reduced. This was demonstrated by Figure 4 where the distribution of copper(II) ion among the three ligands is calculated for a model system containing the metal ion and the three peptides in equimolar concentrations. Figure 4 reveals that the majority of copper(II) ions is bonded to the native peptide fragment at any pH values. This difference probably comes from the secondary interactions of the polar side chains of Asp, Glu, Ser and Arg residues. Moreover, this difference reveals that the amino acid sequence of the N-terminal domains of amyloid peptides is well suited for the complexation with copper(II) ions.

In addition to copper(II) the amyloid-β N-terminus can also effectively bind nickel(II) ions but only mononuclear species can be formed with the exclusive binding from the amino terminus. On the other hand, there is no indication for extra stabilization from the polar

residues and the thermodynamic stability of the nickel(II) complexes corresponds well to those of the common oligopeptides. Zinc(II) ions are not able to promote deprotonation and metal ion coordination of amide nitrogens resulting in a moderate stability of the zinc(II) complexes. This is in accordance with previous findings that the amino terminus is not the preferred zinc(II) binding site of amyloid-β. On the other hand, the comparison of the stability constants of zinc(II) complexes of oligoglycines with that of the native nonapeptide reveals a stability enhancement for the latter one. This enhanced stability is accompanied with a reduced tendency of hydrolysis of zinc(II)-Aβ(1-9) complexes, which can be explained by the simultaneous coordination of terminal aspartyl and histidyl sites in a macrochelate and by the weak secondary interactions of the polar side chains of the other amino acids.

5. Abbreviations

CD – circular dichroism

MS – mass spectra

Fmoc – N-fluorenylmethoxycarbonyl

DMF – dimethyl formamide

TFA – trifluoracetic acid

TIS – triisopropylsilane

Trt – trytil

Pbf – (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)

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Table 1

Protonation constants of the nonapeptides.

T = 298 K, I = 0.2 M

Species	Aβ(1-6) NH_2-DAEFRH-NH_2 [13]	Aβ(1-9) NH_2-DAEFRHDSG-NH_2	NH_2-DAAAAHAAA-NH_2	NH_2-DAAAAAHAA-NH_2
[HL]	7.59	7.73(1)	7.69(1)	7.65(1)
[H_2L]	13.86	14.06(1)	14.01(2)	14.01(1)
[H_3L]	18.04	18.36(2)	16.63(2)	16.83(1)
[H_4L]	20.85	21.83(3)	-	-
[H_5L]	-	24.45(8)	-	-
pK(-NH_2)	7.59	7.73	7.69	7.65
pK(Im)	6.27	6.33	6.32	6.36
pK(-COOH)	4.18	4.30	2.62	2.82
pK(-COOH)	2.81	3.47		
pK(-COOH)	-	2.62		

Table 2

Stability constants (log β_{pqr}) of the copper(II) complexes of the peptides.

T = 298 K, I = 0.2 M

Species	Aβ(1-6) NH_2-DAEFRH-NH_2 [13]	Aβ(1-9) NH_2-DAEFRHDSG-NH_2	NH_2-DAAAAHAAA-NH_2	NH_2-DAAAAAHAA-NH_2
$[CuH_2L]$	17.36	16.94(3)	-	-
[CuHL]	13.16	12.99(2)	12.24(3)	12.23(3)
[CuL]	8.76	8.12(2)	7.66(2)	7.45(2)
$[CuH_{-1}L]$	3.02	1.97(2)	1.60(3)	1.65(2)
$[CuH_{-2}L]$	-4.86	-5.85(2)	-6.73(4)	-6.83(2)
$[CuH_{-3}L]$	-13.50	-14.85(2)	-15.76(4)	-15.94(2)
$[Cu_2H_{-3}L]$	-7.18	-7.94(2)	-9.29(6)	-9.79(4)
$[Cu_2H_{-4}L]$	-14.08	-15.38(2)	-17.86(5)	-18.57(4)
$[Cu_2H_{-5}L]$	-21.55	-22.75(4)	-28.04(6)	-28.00(5)
$[Cu_2H_{-6}L]$	-	-33.41(3)	-	-39.76(3)
pK1	4.20	3.95	-	-
pK2	4.40	4.87	4.58	4.78
pK3	5.74	6.15	6.06	5.80
pK4	7.88	7.82	8.33	8.48
pK5	8.64	9.00	9.03	9.11

Table 3

UV-visible and CD spectroscopic parameters of the major mononuclear complexes formed in the copper(II)- and nickel(II)-nonapeptide systems

Species	Aβ(1-9) NH_2-DAEFRHDSG-NH_2		NH_2-DAAAAHAAA-NH_2		NH_2-DAAAAAHAA-NH_2	
	($\lambda_{max}/\varepsilon$, nm/ $M^{-1}cm^{-1}$)	($\lambda/\Delta\varepsilon$, nm/ $M^{-1}cm^{-1}$)	($\lambda_{max}/\varepsilon$, nm/ $M^{-1}cm^{-1}$)	($\lambda/\Delta\varepsilon$, nm/ $M^{-1}cm^{-1}$)	($\lambda_{max}/\varepsilon$, nm/ $M^{-1}cm^{-1}$)	($\lambda/\Delta\varepsilon$, nm/ $M^{-1}cm^{-1}$)
[CuL]	696/41	-	685/44	-	682/40	-
[$CuH_{-1}L$]	619/94	591/-0.36	618/104	625/-0.35	623/97	621/-0.38
[$CuH_{-2}L$]	560/101	583/-0.38 496/-0.41	566/109	560/-0.35	557/107	554/-0.29
[$CuH_{-3}L$]	514/162	664/0.25 521/-1.57 313/0.72	517/168	699/0.066 523/-0.90 307/0.57	515/167	673/0.057 529/-0.88 309/0.35
[$NiH_{-3}L$]	418/211	469/-3.28 413/- 2.11(sh)	414/247	463/-4.11	414/173	466/-2.54

Table 4

Stability constants (log β_{pqr}) of the nickel(II) complexes of the peptides.

T = 298 K, I = 0.2 M

Species	Aβ(1-6) NH_2-DAEFRH-NH_2 [15]	Aβ(1-9) NH_2-DAEFRHDSG-NH_2	NH_2-DAAAAHAAA-NH_2	NH_2-DAAAAAHAA-NH_2
[NiHL]	10.60	11.31(8)	11.24(11)	11.19(6)
[NiL]	5.63	5.53(4)	5.29(5)	5.31(5)
$[NiH_{-1}L]$	-3.05	-	-3.05(7)	-
$[NiH_{-2}L]$	-	-	-	-
$[NiH_{-3}L]$	-20.00	-20.63(4)	-20.38(6)	-20.74(5)

Table 5

Stability constants (log β_{pqr}) of the zinc(II) complexes of the peptides.

T = 298 K, I = 0.2 M

Species	Aβ(1-9) NH_2-DAEFRHDSG-NH_2	NH_2-DAAAAHAAA-NH_2	NH_2-DAAAAAHAA-NH_2
[ZnHL]	10.09(5)	10.40(4)	10.28(4)
[ZnL]	4.25(1)	4.37(1)	4.11(1)
[ZnH$_{-1}$L]	-4.50(3)	-3.70(1)	-3.38(5)

Legends to Figures

Figure 1

Species distribution of the complexes formed in the copper(II)-Aβ(1-9) system at 1:1 (a) and 2:1 (b) metal to ligand ratios (c_L = 2 mM).

Figure 2

Circular dichroism spectra of the [$CuH_{-3}L$] species of the ligands Aβ(1-9) (a), Aβ(1-4) (b), Ac-Aβ(1-6) (c) and [$Cu_2H_{-6}L$] of Aβ(1-9) (d).

Figure 3

ESI-MS spectra of the 2:1 copper(II)-Aβ(1-9) sample showing the isotopic distribution of the measured (upper) and calculated (lower) spectra for the mononuclear $[CuH_{-1}L]^{2-}$ and dinuclear $[Cu_2H_{-3}L]^{2-}$ species.

Figure 4

Distribution of the copper(II) ion among the three ligands in a model sample containing the metal ion and the three peptides in equimolar concentration (c = 2 mM).

Figure 5

Circular dichroism spectra of the $NiH_{-3}L$ complexes formed with ligands Aβ(1-9) (a), Aβ(1-4) (b) and Ac-Aβ(1-6) (c).

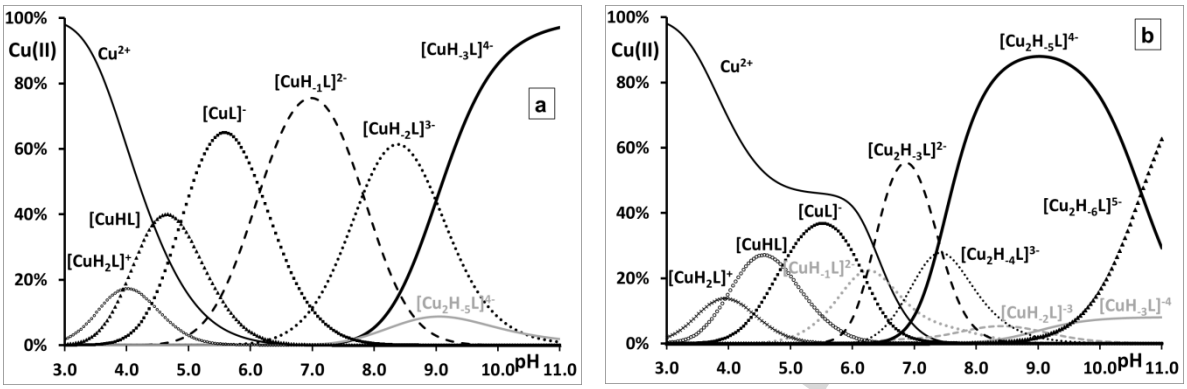


Figure 1

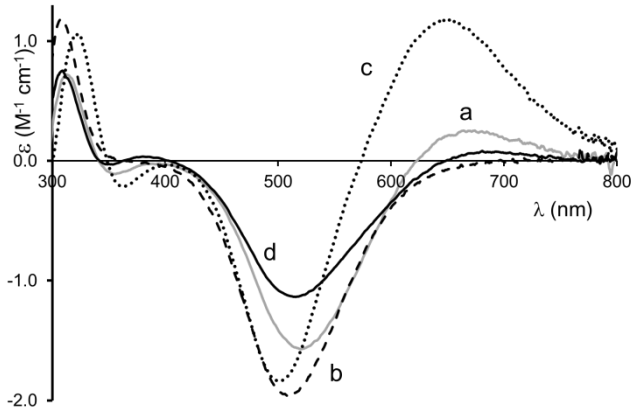


Figure 2

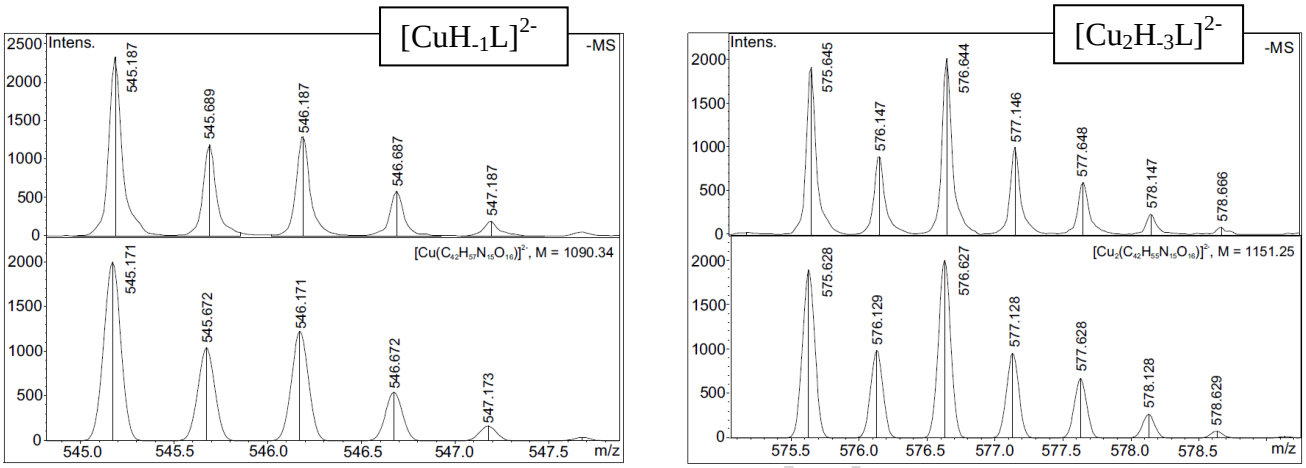


Figure 3

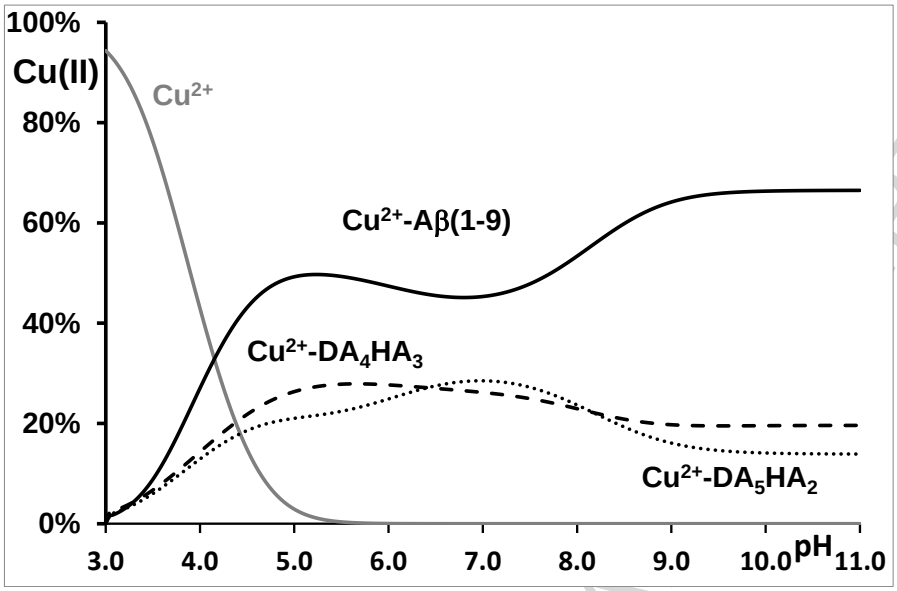


Figure 4

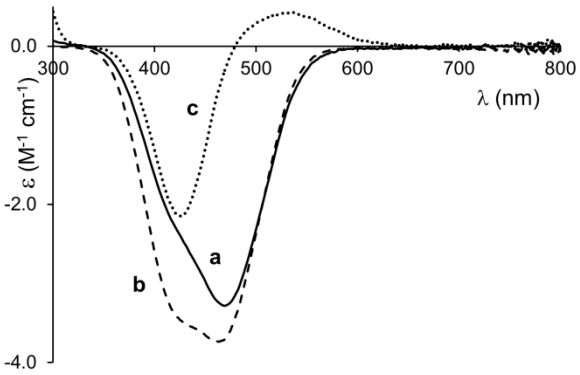
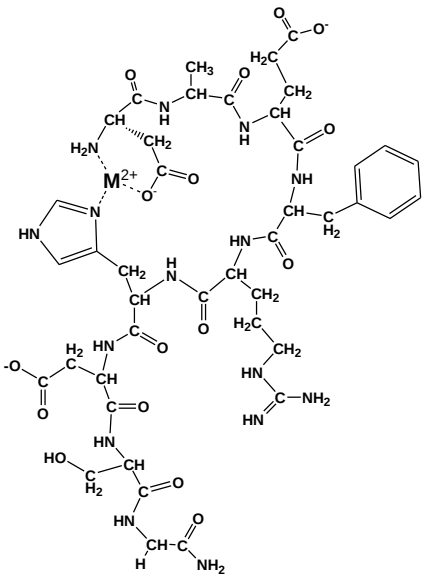
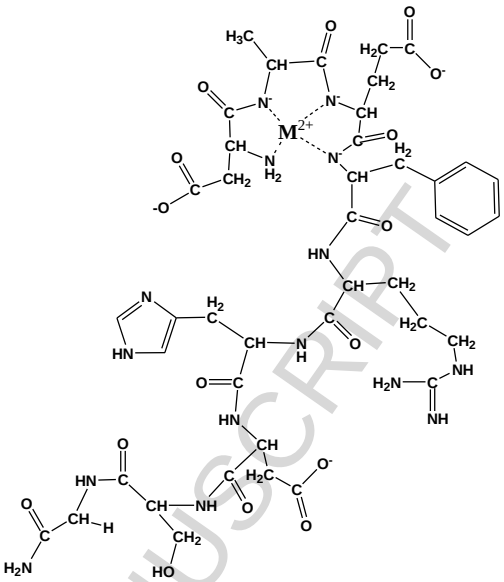


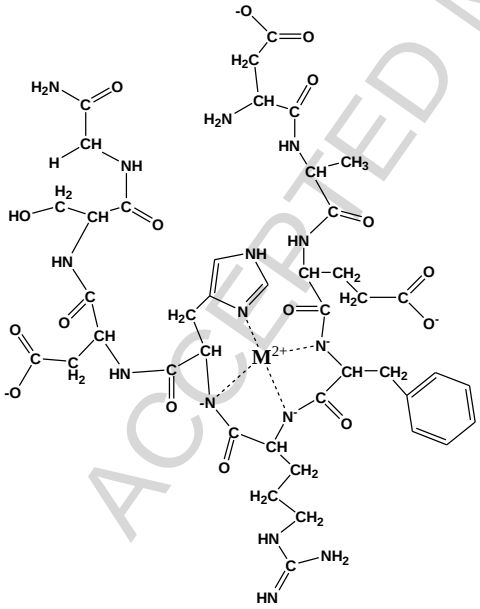
Figure 5



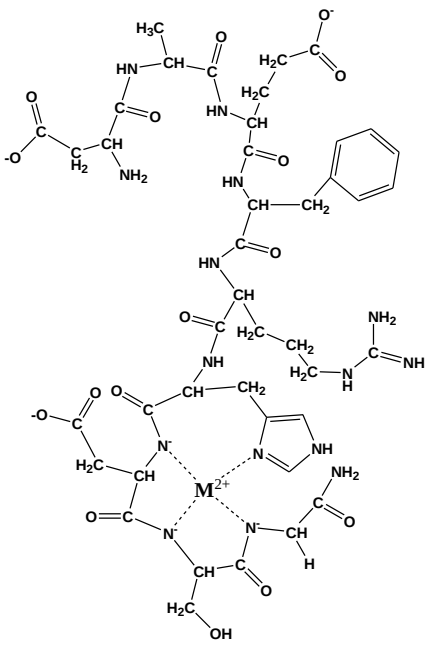
a



b

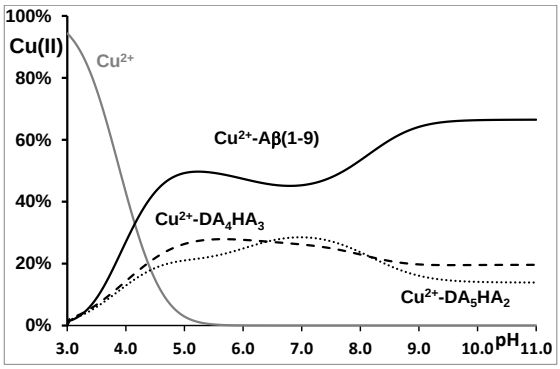


c



d

Scheme 1



Graphical Abstract

Synopsis

The amino acid sequence of the N-terminal domains of amyloid peptides is well suited for the complexation with copper(II) ions.

Highlights

- The amino terminus is the primary ligating group for all peptides.
- Internal His residues enhance the stability of complexes via macrochelation.
- Dinuclear complexes are formed in the copper(II) containing systems.
- The native peptide is a better complexing agent than the alanine mutated derivatives.
- Amino acid sequence of amyloid-β is well suited for complexation with copper(II).