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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.

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. A huge number of papers have 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 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 of 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

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publications [15–18] but some structural details of these interactions remained unanswered. 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-Asp AlaAlaAlaAlaHisAlaAlaAla-NH_2 (DAAAAHAAA) and NH_2-AspAlaAla AlaAlaAlaHisAlaAla-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 × 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 of 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 compared 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 Eqs. (1) and (2):

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

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

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 $c_L = 3.5 \times 10^{-4}$ 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