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Copper(II) and nickel(II) binding sites of peptide containing adjacent histidyl residues

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ABSTRACT

Copper(II) and nickel(II) complexes of the terminally protected nonapeptide Ac-SGAEGHHQK-NH_2 modeling the metal binding sites of the (8–16) domain of amyloid-β have been studied by potentiometric, UV–vis, CD and ESR spectroscopic methods. The studies on the mutants containing only one of the histidyl residues (Ac-SGAEGAHQK-NH_2, Ac-SGAEGHAQK-NH_2) have also been performed. The formation of imidazole and amide coordinated mononuclear complexes is characteristic of all systems with a preference of nickel(II) binding to the His14 site, while the involvement of both histidines in metal binding is suggested in the corresponding copper(II) complexes. The formation of bis(ligand) and dinuclear complexes has also been observed in the copper(II)-Ac-SGAEGHHQK-NH_2 system. The results provide further support for the copper(II) binding ability of the (8–16) domain of amyloid-β and support the previous assumptions that via the bis(ligand) complex formation copper(II) ions may promote the formation of the oligomers of amyloid-β.

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1. Introduction

The development of the various forms of neurodegenerative disorders is associated with protein misfolding and accumulation into amyloid fibrils. Alzheimer's disease (AD) is the most common and best studied among them and it is related to the aggregation of a 39–43 residue peptide called amyloid-β (Aβ). It is also widely accepted that various metal ions are accumulated in the amyloid plaques, especially copper, zinc and iron. Huge number of studies was promoted by this observation to understand the role of metal ions in protein misfolding and to clarify the major metal binding sites of amyloid-β and related substances. A series of specific reviews have already been published in this subject describing the most important findings both on the metallostasis in neurodegeneration and on the coordination chemistry of amyloid peptides [1–10]. From a chemical point of view, the most important observations are associated with the characterization of the major metal binding sites of the peptides. It is clear from the above-mentioned compilations that the N-terminal part of amyloid-β is responsible for metal binding especially for copper(II) and zinc(II) ions.

The amino acid sequence of the peptide supports this conclusion because the N-terminal domain (e.g., Aβ(1–16)) is rich in polar and coordinating side chains, while the amino acids in the internal and C-terminal domains contain mainly apolar side chains promoting the aggregation processes. Therefore, the basic coordination chemical studies were performed mainly for the various N-terminal fragments including Aβ(1–16) and Aβ(1–28) [10–12]. The low solubility of these peptides and their complexes, however, significantly hindered the complete description of the solution equilibria of the metal ion–peptide systems. The PEG-ylation (PEG = polyethylene glycol) helped to overcome this problem and in the last few years our groups published several papers on the copper(II), nickel(II) and zinc(II) complexes of Aβ(1–16)-PEG [13–16]. The amino acid sequence of the hexadecapeptide fragment of amyloid-β is DAEFRHDSGYEVHHQK-NH_2, i.e., it contains three histidyl residues (H6, H13 and H14) and a terminal amino group which has been considered as the primary metal binding site. Moreover, the peptide contains several polar side chains, such as Asp, Glu, Tyr, Ser and Lys residues which can also contribute to metal binding. The combined application of potentiometric and spectroscopic measurements revealed that Aβ(1–16)-PEG can bind as much as four copper(II), three zinc(II) or two nickel(II) ions but some structural details around the primary ligating sites remained unanswered. Our most recent paper on the metal complexes of a C-terminally shortened fragment Aβ(1–9) helped to clarify the exact binding modes around the terminal amino and H6 sites [17]. The characterization of the metal binding around the adjacent histidines (H13 and H14) is, however, more complicated although the previous studies on the Ac-Aβ(8–16)Y10A fragment definitely supported the binding of two copper(II) ions in this domain [13]. It was also clear from these studies that phenolate-O^- of Tyr10 and ε-amino group of Lys16 are not involved in copper(II) binding. The previous experiments, however, did not provide information on the H13/H14 preference in a mononuclear species and on the possible role of the other weakly coordinating side chains (Ser8, Glu11 and Gln15) in metal binding. Moreover,

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some recent studies suggest the preference for the formation of mainly or even solely imidazole coordinated species with the involvement of H13 and H14 sites [18–21]. The formation of these species suggests the presence of bis(ligand) complexes which were not considered in our previous measurements in the equimolar systems.

To answer these questions three mutants of Ac-Aβ(8–16)Y10A have been synthesized and their copper(II) and nickel(II) complexes were studied by potentiometric, UV–vis, CD and EPR spectroscopic methods. In the first peptide Ac-SGAEGHHQK-NH_2 Val12 was replaced by Gly12. There is no any coordinating side chain in valine therefore its replacement with glycine cannot change the coordinating donors but makes it possible to distinguish between the CD spectra of ($3N_{amide}$,N_{im}) coordinated species formed by the involvement of H13 and H14 residues. One of the histidines were substituted by alanine in the other two mutants (Ac-SGAEGAHQK-NH_2 and Ac-SGAEGHAQK-NH_2) providing the appropriate models for the solely H13 or H14 coordinated species. The interaction of nickel(II) ions with amyloid-β is probably not significant biologically but nickel(II)–peptide complexes are often used as structural models for the better understanding of copper(II) complexes [22].

2. Experimental

2.1. Peptide synthesis and other materials

Solid phase peptide synthesis was performed to synthesize all the terminally protected peptides Ac-SGAEGHHQK-NH_2 (Aβ(GHH)), Ac-SGAEGAHQK-NH_2 (Aβ(GAH)) and Ac-SGAEGHAQK-NH_2 (Aβ (GHA)) using a microwave-assisted Liberty 1 Peptide Synthesizer (CEM, Matthews, NC). Introducing of the amino acid derivatives was performed according to the TBTU/HOBt/DIEA activation strategy on the Rink Amide AM resin, using the Fmoc/*t*Bu technique. 30 W microwave power was used for 180 s at 80 °C to remove the Fmoc protecting group by means of 20 V/V % piperidine and 0.1 M HOBt·H_2O in DMF. Coupling of the amino acids was carried out using four times excess of amino acids at 80 °C with 30 W microwave power for 300 s while reacting with 0.5 M HOBt and 0.5 M TBTU in DMF and 2 M DIPEA in NMP as activating reagents. At the end of the synthesis the Fmoc group of the N-terminus of the peptide sequence was removed as described earlier. The free amino group was treated with DMF containing 5 V/V% Ac_2O and 6 V/V% DIEA to perform the acetylated amino terminus. Using a mixture of TFA/TIS/H_2O/2,2′-(ethylenedioxy)diethanethiol (94/2.5/2.5/1 V/V) for 2 h at room temperature the peptides were cleaved from their resins and simultaneously removed their side chain protective groups. Each peptide solved in trifluoroacetic acid was filtered from the resin and precipitated by cold diethyl ether to recover. The solid product was also washed with cold diethyl ether and after separation dried, dissolved in water and finally frozen for lyophilization.

Analytical rp-HPLC analyses were performed using a Jasco instrument equipped with a Jasco MD-2010 plus multi-wavelength detector monitoring the absorbance at 222 nm to check the purity of the synthesized peptides. Isocratic elution with 3% of solvent A was carried out using solvent A (0.1 V/V% TFA in acetonitrile) and solvent B (0.1 V/V% TFA in water) at a flow rate of 0.8 ml/min applying a Vydac C18 chromatographic column (250 × 4.6 mm, 300 Å pore size, 5 μm particle size). The purity of the peptides was greater than 91% for each crude product, confirmed by pH-potentiometric measurements, too, identifying the protonation sites. The ESI-MS measurements in the positive mode showed the exact molecular weight of the $[M + H]^+$ adduct of the three ligands. Amino acid residues and deprotonation processes of the Ac-SGAEGHHQK-NH_2 ligand were confirmed by pH-dependent ^1H NMR spectra (see Supplementary information, Fig. S1).

All solvents and chemicals used for synthesis were obtained from commercial sources in the highest available purity and used without further purification. The N-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (Fmoc-Ser(tBu)-OH, Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Gln(Trt)-OH and Fmoc-Lys(Boc)-OH), 2-(1-H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and the Rink Amide AM resin were purchased from Novabiochem (Switzerland). N,N-diisopropylethylamine (DIEA) and trifluoroacetic acid (TFA) were Merck products, while N-hydroxybenzotriazole (HOBt), N-methyl-pyrrolidone (NMP), triisopropylsilane (TIS), 2,2′-(ethylenedioxy)diethanethiol (DODT) and 2-methyl-2-butanol were from Sigma-Aldrich. Piperidine, acetic acid (AcOH), diethyl ether (Et_2O) and dichloromethane (DCM) were Molar solvents as well as acetonitrile (ACN) and peptide-synthesis grade N,N-dimethylformamide (DMF) and acetic anhydride were purchased from VWR.

Analytical grade reagents of $CuCl_2$ and $NiCl_2$ were used to prepare the metal ion stock solutions and gravimetry was carried out to check their concentrations via the precipitation of oxinates.

2.2. Potentiometric measurements

MOLSPIN pH-meter was applied to carry out the titrations equipped with a 6.0234.100 combination glass electrode (Metrohm) and a MOL-ACS microburette controlled by a computer. The pH-potentiometric measurements were carried out at 298 K and at a constant ionic strength of 0.2 M KCl using carbonate-free stock solution of potassium-hydroxide with a known exact concentration of around 0.2 M. Argon gas was introduced above the solutions during the titrations to ensure the absence of carbon dioxide and dioxygen and a VELP Scientific magnetic stirrer was used to homogenize the samples. Ligand concentration was set in the range of 1.5×10^{-3}–2×10^{-3} M in the titrated samples with the initial volume of 3 cm^3 and the metal to ligand ratios were 1:2, 1:1 and 2:1 for the Aβ(GHH) derivative while 1:2 and 1:1 for the Aβ(GAH) and Aβ(GHA) peptides. Corrections have been made to eliminate the diffusion potential by determining and subtracting the Irving factor from the measured pH values [23]. The recorded pH readings were converted to hydrogen ion concentration. Protonation constants of the ligands and overall stability constants (log β_{pqrs}) of the metal complexes were calculated by means of the general computational programs (PSEQUAD and SUPERQUAD) as described in our previous publications [24,25]. Eqs. (1) and (2) define the equilibrium constants for the binary systems.

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

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

2.3. Spectroscopic measurements

The same concentration range as used for pH-potentiometry was selected to register the UV–vis spectra of the copper(II) and nickel(II) complexes. A Perkin Elmer Lambda 25 scanning spectrophotometer was applied in the wavelength range of 250–800 nm for copper(II)- and 250–1100 nm for nickel(II) complexes.

A JASCO J-810 spectropolarimeter was used to record the CD spectra of the same complexes from 280 to 800 nm using 1 and/or 10 mm cells and the same concentrations as used for pH-potentiometric measurements. Resolution of the UV–visible and CD-spectra was performed by using the CCA+ computational program in the case of simultaneously present copper(II) complexes [26].

In case of copper(II) complexes frozen solution ESR measurements were carried out at 120 K on a Bruker EMX spectrometer, equipped with a HP 53150A microwave frequency counter. Metallic copper was purchased from JV Isoflex (Moscow, Russia) containing 99.3% ^{63}Cu and 0.7% ^{65}Cu and was converted into the sulfate. ESR spectra have better resolution using $CuSO_4 \cdot 5H_2O$ that had been enriched with ^{63}Cu, of which the copper(II) stock solutions were prepared. Increasing the resolution and avoid the aggregation process 10% ethylene glycol was added to the aqueous copper(II) complex samples.

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