pH-dependence of the specific binding of Cu(II) and Zn(II) ions to the amyloid-β peptide

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ABSTRACT

Metal ions like Cu(II) and Zn(II) are accumulated in Alzheimer's disease amyloid plaques. The amyloid-β (Aβ) peptide involved in the disease interacts with these metal ions at neutral pH via ligands provided by the N-terminal histidines and the N-terminus. The present study uses high-resolution NMR spectroscopy to monitor the residue-specific interactions of Cu(II) and Zn(II) with ^{15}N- and ^{13}C,^{15}N-labeled Aβ(1–40) peptides at varying pH levels. At pH 7.4 both ions bind to the specific ligands, competing with one another. At pH 5.5 Cu(II) retains its specific histidine ligands, while Zn(II) seems to lack residue-specific interactions. The low pH mimics acidosis which is linked to inflammatory processes in vivo. The results suggest that the cell toxic effects of redox active Cu(II) binding to Aβ may be reversed by the protective activity of non-redox active Zn(II) binding to the same major binding site under non-acidic conditions. Under acidic conditions, the protective effect of Zn(II) may be decreased or changed, since Zn(II) is less able to compete with Cu(II) for the specific binding site on the Aβ peptide under these conditions.

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1. Introduction

Despite more than a decade of intense studies, the roles of metal ions in the process of aggregation and amyloid formation by the amyloid-β (Aβ) peptide involved in Alzheimer's disease are still not well understood. Early studies [1] showed that Cu(II), Zn(II) and Fe(III) ions give rise to increased pH dependent aggregation of Aβ(1–40), suggesting that aggregation is strongly induced by conditions representing physiological acidosis. Recent work [2] on the kinetics of fibril (amyloid) formation (determined from kinetic studies of thioflavin T fluorescence) have shown that low pH and Cu(II) ions could strongly accelerate the kinetics of fibril formation, whereas Zn(II) simply inhibited the formation of fibrils.

Other recent studies have shown distinct pathways for Aβ aggregation in the presence of Cu(II) ions at neutral pH for different metal-to-peptide ratios [3], whereas Zn-induced aggregation of Aβ has been proposed to give rise to new kinds of aggregated assemblies [4]. A recent review describes the present understanding of the Aβ peptide interactions with Cu(II) and Zn(II) and their effects on the fibrillation and toxicity at physiological conditions [5].

Given the proposed connection between metal ions and Aβ amyloid formation, metal chelation therapy has been tried and evaluated as a potential therapy against Alzheimer's disease [6]. However, the understanding is limited and the results are not conclusive, showing the need for further studies of the metal binding properties of the Aβ peptide [7].

Various specific ligands to the metal ions have been proposed [8,9], and our previous NMR studies of Aβ(1–40) at pH 7.4 have shown a major binding site, common for Zn(II) and Cu(II), with H6, H13, H14 and the N-terminus as ligands [10,11]. In the same study [10] we reported apparent dissociation constants in the micromolar range, with a somewhat higher Cu(II) affinity, 0.4 μM, than Zn(II) affinity, 1.1 μM (both values not corrected for ionic strength, cf. [5,12]). Both the histidine ligandation and the somewhat higher stability of the Cu(II) complex than the Zn(II) complex are supported by theoretical calculations [13].

Here we have studied the residue-specific interactions of Cu(II) and Zn(II) with Aβ(1–40) at three different pHs using high resolution NMR spectroscopy and ^{15}N- and ^{13}C,^{15}N-labeled Aβ(1–40) peptide. In agreement with our previous study, at pH 7.4 both ions bind to the specific ligands, competing with one another and displaying similar binding affinities. In contrast, at pH 5.5 only Cu(II) retains its specific histidine binding ligands, while Zn(II) seems to lose its residue specificity in the interactions. Since Cu(II) binding and acidosis linked to inflammatory processes in vivo have been suggested to enhance the neurotoxicity of Aβ [5], our observations suggest a rational molecular explanation for these effects: Under non-acidic conditions the toxic effects of redox active Cu(II) binding to Aβ

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(involving e.g. formation of Reactive Oxygen Species (ROS)) [14] may be attenuated by the protective activity of non-redox active Zn(II) binding to the same major binding site. In contrast, acidic conditions would decrease or change the protective effect of Zn(II), which is less able to compete with Cu(II) for the specific binding site on Aβ at low pH.

2. Materials and methods

2.1. Sample preparation

Unlabeled, ¹⁵N-labeled and ¹³C,¹⁵N-labeled Aβ(1–40) were bought from AlexoTech (Umeå, Sweden) and prepared as previously described [15,16] with some modifications. First the peptide was dissolved in 10 mM NaOH to a peptide concentration of 1 mg/ml. After 1 min of sonication in ice bath, chilled double distilled water was added to half the final sample volume. After another sonication step sodium phosphate buffer (for samples of pH 7.4 and 6.5) or sodium acetate buffer (for samples of pH 5.5) was added to a final concentration of 10 mM. However, at pH 5.5 we are very close to the pI of the peptide (pI ≈ 5.5) [16], which made it difficult to obtain a stable sample, especially with the ¹³C,¹⁵N-labeled peptide. Thus, an alternative protocol was developed where the ¹³C,¹⁵N-labeled Aβ(1–40) peptide was dissolved in 5 mM DCl (Larodan AB, Malmö, Sweden), sonicated in an ice bath for 1 min and mixed with deuterated sodium acetate buffer (10 mM final buffer concentration) based on acetic-d₃ acid-d (Sigma, St. Louis, MO, USA). The pH was adjusted to 5.5 with 10 mM NaOD (Merck, Darmstadt, Germany). This protocol yielded a more stable Aβ sample during the preparation, and the deuterated solutions were used to avoid the water signal in the ¹H–¹³C HSQC spectra. In order to minimize aggregation, sample preparation was always carefully performed on ice.

2.2. NMR spectroscopy

Bruker Avance 500 and 700 MHz spectrometers were used to record NMR spectra of 75 μM isotope-labeled Aβ(1–40) peptide at 5 °C. Both machines were equipped with triple-resonance cryogenically cooled probeheads, and all spectra were referenced according to the water signal and analyzed in nmrPipe and Sparky [17,18].

The 500 MHz NMR spectrometer was used to record ¹H–¹⁵N heteronuclear single quantum correlation (HSQC) spectra when 10, 20, 30, 40 and 50 μM copper chloride or zinc acetate was titrated to ¹⁵N-labeled Aβ(1–40) peptide dissolved in 10 mM phosphate buffer at pH 7.4 and 6.5, or dissolved in 10 mM sodium acetate buffer at pH 5.5 (90/10 H₂O/D₂O).

The 700 MHz NMR spectrometer was used to various assignment experiments, and to record ¹H–¹³C HSQC spectra when 5, 10, 15, 20, 25 and 30 μM copper chloride was titrated to ¹³C,¹⁵N-labeled Aβ(1–40) peptide dissolved in 10 mM phosphate buffer at pH 7.4 and 6.5, or dissolved in 10 mM acetate buffer at pH 5.5 (100% D₂O).

We have previously published the assignment of the ¹H–¹⁵N HSQC spectrum of Aβ(1–40) at pH 7.4 [19]. Using this known assignment it was straightforward to obtain the assignment of the ¹H–¹⁵N HSQC cross-peaks of Aβ(1–40) at pH 6.5 and 5.5 by following the chemical shift changes at decreasing pH. To assign additional peaks, i.e. A2 and the histidines, NOESY–HSQC (¹H–¹⁵N), TOCSY–HSQC (¹H–¹⁵N), HNCA and HN(CO)CA experiments were carried out. ¹H–¹³C cross-peaks at pH 5.5 were assigned by HNCA and HN(CO)CA together with the assignment at pH 7.4 communicated by Zagorski [20]. For the quantitation of spectra, the cross-peak amplitudes were evaluated.

2.3. CD spectroscopy

A Chirascan CD unit from Applied Photophysics was used to monitor each titration step when copper and zinc acetate was titrated to a 20 μM solution of Aβ(1–40) peptide dissolved in either 20 mM sodium phosphate buffer at pH 7.4 or 20 mM sodium acetate buffer at pH 5.5. The sample was held in a 2 mm pathlength quartz cuvette with 400 μl sample volume. CD spectra were recorded at 25 °C in the region 190–270 nm using a step size of 2.5 nm and a 1 nm slit size. Zinc and copper acetate was added in metal-to-Aβ-peptide molar ratios of 1:2, 2:2, 3:2, 5:2, 8:2, 13:2, 19:2, 30:2, and 50:2.

3. Results

Two-dimensional ¹H–¹⁵N- and ¹H–¹³C HSQC spectra were recorded at 5 °C for respectively ¹⁵N- and ¹³C,¹⁵N-labeled Aβ(1–40) peptides at the three pH values 5.5, 6.5, and 7.4. Titrations with copper and zinc were found to reduce the peak intensities in the HSQC spectra, and the patterns of peak intensity reduction varied between the two metal ions and between the three different pH values.

Fig. 1A–C shows the ¹H–¹⁵N HSQC spectra of 75 μM ¹⁵N-labeled Aβ(1–40) alone and in the presence of 30 μM copper chloride at 5 °C at pH 7.4, 6.5 and 5.5. Fig. 2A–C shows the intensities of the NH cross-peaks along the peptide sequence at these pHs. At pH 7.4 the cross-peak intensities corresponding to the residues near the N-terminus of Aβ(1–40), i.e. residues 1–17, are around 20% percent of the peak intensities without copper. This is consistent with previous results indicating that metal ions bind to histidines 6, 13, and 14 of Aβ(1–40) [10]. Also the protons belonging to the central and C-terminal residues of Aβ(1–40) display reduced intensities, around 60–70% of the peak intensities without copper. There are no obvious chemical shift changes accompanying the loss of signal intensities. For pH 6.5 a similar pattern can be seen, although the intensity ratios of the amide protons are below 10% near the N-terminus and around 20–30% for the central and C-terminal residues. For pH 5.5 the same intensity pattern as for pH 6.5 is observed. Thus, for all three studied pH values there appears to be specific binding of copper ions near the N-terminus, resulting in specific loss of ¹H–¹⁵N cross-peak intensities in this part of the sequence.

Similar results were obtained in the ¹H–¹³C HSQC spectra for the cross-peaks from Cᵅ–H in the ¹³C,¹⁵N-labeled Aβ(1–40) (Supplementary Figs. S1 and S2). At pH 7.4 a major reduction in peak intensity is seen for the N-terminal residues after addition of copper, while a smaller peak intensity reduction is seen for the central and C-terminal residues. At pH 5.5 all Cᵅ–H cross-peak intensities are reduced to around 5% of their original intensity after addition of copper. It should be mentioned that the Aβ samples slightly aggregated during the titrations, and this aggregation was more pronounced at low pH. The ¹³C,¹⁵N-labeled sample was particularly prone to aggregate.

Fig. 1D–F shows the ¹H–¹⁵N HSQC spectra of 75 μM ¹⁵N-labeled Aβ(1–40) alone and in the presence of 30 μM zinc acetate at 5 °C at pH 7.4, 6.5 and 5.5. Fig. 2D–F shows the ¹H–¹⁵N HSQC cross-peak intensities at these pHs. At pH 7.4 the results are similar to those of the copper addition – i.e. significant signal loss of amide cross-peak intensities for residues 1–16, and a smaller loss of signal – around 80% remaining – for the central and C-terminal residues 17–40. At pH 6.5 the specific intensity loss of cross-peaks from residues at the N-terminus is less pronounced. At pH 5.5 the cross-peak intensity for all residues is reduced to around 70% of the original intensity after zinc addition. This overall signal intensity ratio at pH 5.5 is much higher than the overall signal intensity ratio after addition of copper, which is about 15% at pH 5.5. At pH 6.5 the overall amide proton intensity ratio is approximately intermediate