Histidine-13 Is a Crucial Residue in the Zinc Ion-Induced Aggregation of the Aβ Peptide of Alzheimer's Disease[†]

Su-Ting Liu,[‡,§] Geoffrey Howlett,[§] and Colin J. Barrow*[,‡]

School of Chemistry and Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, Victoria 3052, Australia

Received January 27, 1999; Revised Manuscript Received April 7, 1999

ABSTRACT: Metal ions such as Zn^{2+} and Cu^{2+} have been implicated in both the aggregation and neurotoxicity of the β-amyloid (Aβ) peptide that is present in the brains of Alzheimer's sufferers. Zinc ions in particular have been shown to induce rapid aggregation of Aβ. Rat Aβ binds zinc ions much less avidly than human Aβ, and rats do not form cerebral Aβ amyloid. Rat Aβ differs from human Aβ by the substitution of Gly for Arg, Phe for Tyr, and Arg for His at positions 5, 10, and 13, respectively. Through the use of synthetic peptides corresponding to the first 28 residues of human Aβ, rat Aβ, and single-residue variations, we use circular dichroism spectroscopy, sedimentation assays, and immobilized metal ion affinity chromatography to show that the substitution of Arg for His-13 is responsible for the different Zn^{2+}-induced aggregation behavior of rat and human Aβ. The coordination of Zn^{2+} to histidine-13 is critical to the zinc ion induced aggregation of Aβ.

Alzheimer's disease (AD) is characterized pathologically by the deposition of amyloid, the major component of which is a 40−42 residue peptide known as the β-amyloid peptide (Aβ) (*1*). Aβ is proteolytically derived from a ubiquitous cell surface protein called the amyloid precursor protein (APP) (*2*). The deposition of amyloid appears to be a critical factor in the development of AD, and Aβ itself has been reported to be neurotoxic (*3*−*6*). Neurotoxicity appears to require Aβ to be in the form of β-sheet aggregates (*7*−*9*), although the degree of aggregation of the neurotoxic species is unknown (*10*, *11*).

Aβ is present in AD brain in both soluble and insoluble forms (*12*−*14*) and interacts with a variety of materials. Amyloid found in AD brain is complexed with several proteins, including apolipoprotein E (apoE) (*15*), APP (*16*) and heparin sulfate proteoglycan (*17*). ApoE has been proposed to act as an antiaggregation agent (*18*, *19*), while this and other proteins have also been suggested to facilitate the conversion of soluble Aβ to the toxic aggregates and fibrils (*20*, *21*). Other factors that influence the aggregation state of Aβ in vivo include pH (*22*−*24*), peptide concentration (*22*, *25*), incubation time (*26*), salt concentration (*26*), glycation (*27*), and the presence of certain metal ions (*28*−*30*).

Zinc and copper ions have been implicated in both Aβ aggregation and neurotoxicity. Aβ preferentially and saturably binds Zn^{2+} (*28*), and Zn^{2+} induces synthetic Aβ aggregation in a pH-dependent and reversible manner (*31*). Zn^{2+} also selectively induces the rapid aggregation of endogenous Aβ in cerebrospinal fluid (CSF). In addition, a possible mechanism for Aβ toxicity is free radical-induced oxidative damage initiated by a metal ion−Aβ-induced Fenton reaction (*32*). High concentrations of zinc have been found in neurones in regions of the brain that are vulnerable in AD, such as cortex and the hippocampus (*33*). Up to 300 μM Zn^{2+} is released into the extracellular space during neurotransmission in the hippocampus (*34*, *35*), which may be sufficient to induce aggregation of Aβ. After transient forebrain ischemis, which has been associated with amyloid deposition, degenerating neurons show a large increase in chelatable Zn^{2+} (*36*, *37*). The Zn^{2+} binding site on Aβ is unknown, although it appears to be between the residues 6 and 28 (*38*). Because Zn^{2+} binding is abolished by acidic pH and by chemical modification of the histidines with diethyl pyrocarbonate, then one or more histidine side chains are probably involved in binding (*29*).

It has been reported that rats do not form cerebral Aβ amyloid (*39*, *40*). In addition, rat Aβ binds Zn^{2+} much less avidly and is less susceptible to Zn^{2+}-induced aggregation than is the human Aβ sequence (*28*). Rat Aβ differs from human Aβ by the substitutions of Gly for Arg, Phe for Tyr, and Arg for His at positions 5, 10, and 13, respectively. The difference in Zn^{2+} binding, aggregation, and amyloid formation of rat and human Aβ and the fact that two of the three substitutions are within the putative N-terminal Zn^{2+} binding region indicate that at least one of these three substituted residues is crucial to Zn^{2+} binding and amyloid formation. Metals such as Cu^{2+} and Ni^{2+} were shown to displace radiolabeled zinc ions ($^{65}Zn^{2+}$) from Aβ, indicating that the binding site is similar for these three metals (*41*), although this study was performed in the absence of a blocking transition metal ion to abolish low-affinity metal ion interactions. Therefore, at least one of the human Aβ residues Arg-

[†] This work was funded by the Australian Research Council and by a SUNBOR grant from the Suntory Institute for Bioorganic Research.

* Correspondence should be addressed to this author: Phone +61-3-9344-6484; Fax +61-3-9347-5180; E-mail c.barrow@chemistry.unimelb.edu.au.

[‡] School of Chemistry.

[§] Department of Biochemistry and Molecular Biology.

10.1021/bi990205o CCC: $18.00
Published on Web 06/30/1999

^{1}DAEFRHDSGYEVHHQKLVFFAEDVGSNK^{28}	hAβ(1-28)
^{1}DAEFGHDSGEEVRHQKLVFFAEDVGSNK^{28}	rAβ(1-28)
^{1}DAEFGHDSGYEVHHQKLVFFAEDVGSNK^{28}	^{5}G-hAβ(1-28)
^{1}DAEFRHDSGEEVHHQKLVFFAEDVGSNK^{28}	^{10}F-hAβ(1-28)
^{1}DAEFRHDSGYEVRHQKLVFFAEDVGSNK^{28}	^{13}R-hAβ(1-28)

FIGURE 1: Sequence of the synthesized peptides.

5, Tyr-10, or His-13 may be critical to the metal binding of both Zn^{2+} and Cu^{2+} ions and in amyloid formation. Iodination of Aβ tyrosine has been suggested to attenuate Zn^{2+}-mediated precipitation (*28*), indicating that this residue may be important for metal binding and aggregation. To test the importance of each rat substitution on metal binding and aggregation we have synthesized a series of Aβ(1−28) peptides based on the human sequence with individual rat substitutions, in addition to the rat [rAβ(1−28)] and human [hAβ(1−28)] peptides (Figure 1). Aβ(1−28) should be a good model for Aβ metal binding because the N-terminal 28 residues of Aβ encompass the putative 6−28 metal binding site, contain all the potentially charged Aβ residues, modulate the conformational behavior and pH dependence of the Aβ sequence (*22*−*24*), and contain all the human to rat substitutions. In addition, hAβ(1−28) has been shown to form amyloid fibrils in vitro (*42*, *43*). Therefore, this sequence should mimic the metal-binding properties of full-length Aβ.

MATERIALS AND METHODS

Peptide Synthesis. Peptides were synthesized by use of manual solid-phase Boc amino acid chemistry with in situ neutralization (*44*). Acylations were performed with 5 equiv of the Boc-protected amino acid, 4.9 equiv of HBTU, 5 equiv of HOBt, and 7.5 equiv of DIPEA. Each acylation was monitored by ninhydrin monitoring (*45*), and couplings were repeated if necessary. Peptides were cleaved from the resin by anhydrous hydrogen fluoride with *p*-cresol as a scavenger. After hydrogen fluoride removal, peptides were dissolved in trifluoroacetic acid (TFA) and precipitated with ether. Peptides were purified with an acetonitrile/water (0.01%TFA) gradient on a reverse-phase semipreparative Vydac HPLC column. Peptide purity and identity were confirmed by analytical HPLC, electrospray mass spectrometry, and amino acid analysis.

Circular Dichroism Spectroscopy. Circular dichroism (CD) spectra were obtained under a variety of solution conditions using a CD spectropolarimeter, model 62DS (Aviv, Lakewood, NJ). The method used was similar to that previously described for Aβ (*23*). Briefly, a stock solution of each peptide in acetonitrile and water (1:1) was prepared. The concentrations of the stock solutions were determined by quantitative amino acid analysis. A known amount of this stock solution, normally 7 μL, was injected into 300 μL of the appropriate CD buffer directly in the 1 mm path length quartz cell. The solution was mixed and allowed to stand for 5 min before the spectrum was acquired. Measurements were taken at 0.5 nm steps over a 190−250 nm wavelength range, the baseline was subtracted, and the resulting spectra were smoothed. For temperature denaturing studies, the temperature was controlled by a circulating water bath and spectra were recorded at 10 °C intervals from 10 to 60 °C.

Sedimentation Assay. The proportion of pelletable peptide was determined by a modified literature method (*46*, *47*). Briefly, samples were prepared from peptide stock solution by dilution with 20 mM sodium phosphate buffer (pH 7.3) containing 150 mM NaCl. For metal-induced aggregation experiments, metal ions were added from a 0.1 M metal stock solution (Ni^{2+}, Zn^{2+}, or Fe^{3+}). The final peptide concentrations were 100 μM, containing 0−2000 μM metal ions with a total volume of 120 μL. Samples were incubated for 0−24 h at 37 °C, after which insoluble aggregates were removed by centrifugation at 13000*g* for 10 min. The ratio of peptide concentrations, as determined by fluorescamine assay, of the supernatant relative to noncentrifuged peptide was used to calculate the levels of sedimented peptide (*47*). For the fluorescamine assay a Molecular Devices f-max fluorescence microplate reader with a 355 nm excitation wavelength filter and a 460 nm emission filter was used. All experiments were performed in triplicate with 200 μL samples in 96 well plates. Control experiments were performed in parallel without the addition of metal ions and/or centrifugation.

High-Performance Immobilized Metal Ion Affinity Chromatography. High-performance immobilized metal ion affinity chromatography (HP-IMAC) was performed by a modified literature method (*48*, *49*). HiTrap chelating Sepharose cartridges were obtained from Pharmacia and mated to a Beckman HPLC system comprising a System Gold programmable solvent module 126, a diode array detector module 168, and a Rheodyne injector. Peptide was eluted with a pH gradient and two buffers; buffer A, 20 mM potassium phosphate containing 0.5 M NaCl, pH 7.3; buffer B, 20 mM potassium phosphate containing 0.5 M NaCl, pH 4.0. Peptides were freshly prepared in buffer A and concentrations were determined by quantitative fluorescamine assay (*50*). The column was equilibrated with buffer A and charged with a 0.1 M solution of either $NiCl_2$, $ZnCl_2$, or $CuCl_2$ in buffer A until saturated. The excess metal ions were removed by a wash with distilled water and the column was reequilibrated with buffer A. Peptide was eluted with a gradient of 100% buffer A (pH 7.3) to buffer B (pH 4.0) over 15 min at 1 mL/min. Between runs, the column was removed, regenerated by washing manually with distilled water, 50 mM EDTA, and distilled water, and then reequilibrated in buffer A.

RESULTS

Secondary Structure of hAβ(1−28) and rAβ(1−28) in the Absence of Metal Ions. In aqueous solution at pH 7.3 both hAβ(1−28) and rAβ(1−28) exhibit CD spectra consistent with random coil (Figure 2). Both peptides are random coil at pH values from pH 3 to 10 and remain random coil after 24 h (data not shown). In the hydrophobic solvent trifluoroethanol (TFE), which resembles a membrane environment (*51*), both peptides adopt significant α-helical structure (Figure 2). On the basis of the molar ellipticity at 222 nm, the helical content of hAβ(1−28) and rAβ(1−28) in TFE is 48% and 47%, respectively (*52*) In solutions of 10%, 20%, 30%, 40%, 60%, and 90% TFE in buffer at both pH 7.3 and 5.0, the helical contents of both hAβ(1−28) and rAβ(1−28) were identical within experimental error (results not shown). This indicates that the helical propensity is similar for both peptides. Helical denaturing experiments performed at pH 7.3 in 60% TFE for both hAβ(1−28) and rAβ(1−28)

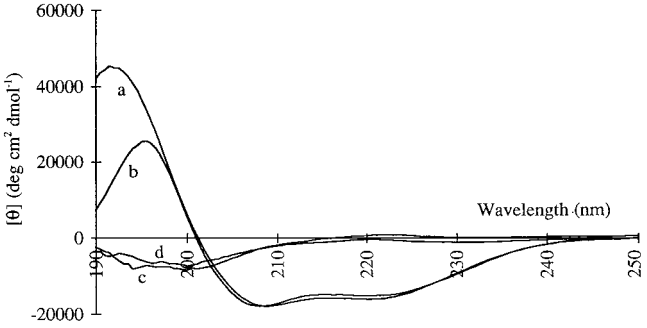

FIGURE 2: CD spectra of hAβ(1−28) and rAβ(1−28) in aqueous buffer at pH 7.3 and in TFE. Spectra labels correspond to (a) hAβ(1−28) in TFE; (b) rAβ(1−28) in TFE; (c) hAβ(1−28) in 10 mM sodium phosphate buffer at pH 7.3; and (d) rAβ(1−28) in 10 mM sodium phosphate buffer at pH 7.3.

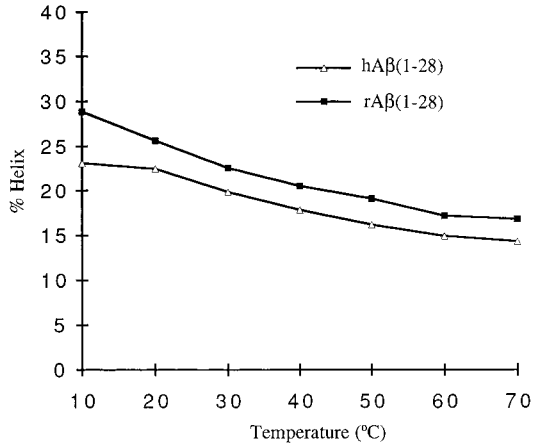

FIGURE 3: Temperature denaturing curves for hAβ(1−28) and rAβ(1−28) in 60% TFE in phosphate buffer at pH 7.3. Helical content for each peptide was determined from the molar ellipticity at 222 nm from CD spectra obtained at temperatures from 10 to 70 °C.

indicate that these peptides have indistinguishable helical stability (Figure 3). For the temperature range from 10 to 70 °C the slope of the temperature denaturing curve was identical within experimental error for both peptides. These results indicate that both hAβ(1−28) and rAβ(1−28) are random coil in aqueous solution and have similar helical content and stability in a hydrophobic environment such as TFE. No β-sheet structure or aggregation was observed for these peptides at CD concentrations in the absence of metal ions.

Metal-Induced Aggregation of hAβ(1−28) and rAβ(1−28). Incubation of hAβ(1−28) or rAβ(1−28) at concentrations of 10 and 100 μM at pH 7.3 for 1 week showed less than 10% aggregation for both peptides at both concentrations. Aggregation was determined by centrifugation followed by fluorescamine assay determination of soluble versus total peptide. To determine the influence of different metal ions on peptide aggregation, hAβ(1−28) and rAβ(1−28) at concentrations of 100 μM in pH 7.3 phosphate buffer were incubated separately with 500 μM Zn^{2+}, Ni^{2+}, or Fe^{3+} for 24 h. Controls containing no added metal ions were also incubated for the same period. All metals caused significant peptide aggregation, with a significant difference being observed between hAβ(1−28) and rAβ(1−28) in the presence of Zn^{2+} (Figure 4). hAβ(1−28) showed 80% aggregation in the presence of Zn^{2+}, while rAβ(1−28) only exhibited 35% aggregation.

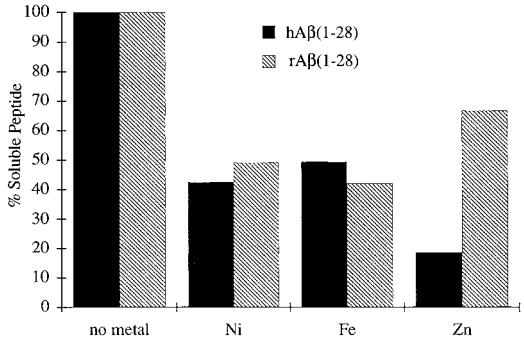

FIGURE 4: Metal-induced aggregation of hAβ(1−28) and rAβ(1−28). Peptides were prepared by diluting peptide stock solutions into 20 mM sodium phosphate buffer at pH 7.3, containing 150 mM NaCl. Peptide solutions (100 μM) containing 500 μM either Ni^{2+}, Fe^{3+}, or Zn^{2+} were incubated for 1 h and then centrifuged at 13000*g* for 10 min. Peptide concentrations, relative to uncentrifuged control without metal, were determined by fluorescamine assay. All data points are means ± SD, $n = 3$.

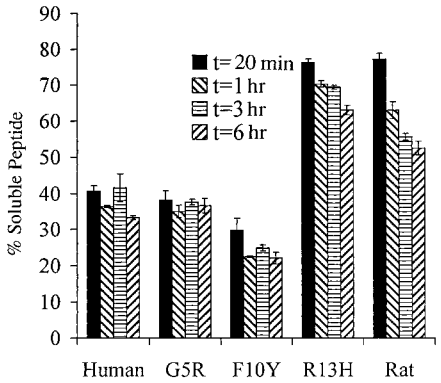

FIGURE 5: Zinc ion-induced time-dependent aggregation of hAβ(1−28), rAβ(1−28), ^{13}R-Aβ(1−28), ^{5}G-hAβ(1−28), and ^{10}F-hAβ(1−28). Peptides were prepared by diluting peptide stock solutions into 20 mM sodium phosphate buffer at pH 7.3, containing 150 mM NaCl. Peptide solutions (100 μM) containing Zn^{2+} (500 μM) were incubated for between 20 min and 24 h and centrifuged at 13000*g* for 10 min. Peptide concentrations, relative to uncentrifuged controls without Zn^{2+}, were determined by fluorescamine assay. All data points are means ± SD, $n = 3$.

Effect of Individual Substitutions on the Zinc Ion-Induced Aggregation of hAβ(1−28). Because of the importance of Zn^{2+}−Aβ interactions in AD and the clear difference observed for hAβ(1−28) and rAb(1−28) with Zn^{2+}, we explored time-dependent Zn^{2+}-induced aggregation of hAβ(1−28), rAβ(1−28), ^{5}G-hAβ(1−28), ^{10}F-hAβ(1−28), and ^{13}R-hAβ(1−28). Each peptide was incubated at pH 7.3 for periods from 20 min to 6 h in the presence of Zn^{2+}. The hAβ(1−28) peptide and the two peptides containing the individual substitutions R to G [^{5}G-hAβ(1−28)] and Y to F [^{10}F-hAβ(1−28)] showed 60−70% aggregation after 20 min with a slight increase in aggregation over 6 h (Figure 5). In contrast, both rAβ(1−28) and the peptide containing the H to R substitution [^{13}R-hAb(1−28)] both showed approximately 23% aggregation after 20 min with an increase to 47% and 36% aggregation after 6 h, respectively. Both rAβ(1−28) and ^{13}R-hAβ(1−28) aggregated significantly less than the other peptides, indicating that the H to R substitution is responsible for the observed difference between hAβ(1−28) and rAβ(1−28) in Zn^{2+}-induced aggregation. Histidine-13 is probably a coordination site for the binding of Zn^{2+} by hAβ(1−28).

Table 1: Elution pH of Each of the Peptides hAβ(1−28), rAβ(1−28), ^{13}R-Aβ(1−28), ^{5}G-hAβ(1−28), and ^{10}F-hAβ(1−28) on Immobilized Iminodiacetate−Metal Chelating Chromatography[a]

	hAβ(1−28)	rAβ(1−28)	^{13}R−Aβ(1−28)	^{5}G-hAβ(1−28)	^{10}F-hAβ(1−28)
IDA−Fe^{3+}	7.3	7.3	7.3	7.3	7.3
IDA−Zn^{2+}	4.0	7.3	7.3	4.0	4.0
IDA−Ni^{2+}	4.0	5.0	5.3	4.0	4.0
IDA−Cu^{2+}	4.0	4.0	4.0	4.0	4.0

[a] Elution at pH 7.3 indicates no affinity, while at pH 4.0 both the peptide and metal are stripped off the column, indicating high affinity. Intermediate elution pHs indicate moderate affinity.

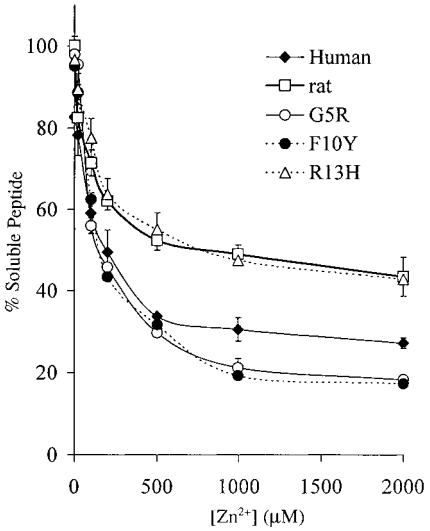

FIGURE 6: Zinc ion concentration dependence of the aggregation of hAβ(1−28), rAβ(1−28), ^{13}R-Aβ(1−28), ^{5}G-hAβ(1−28), and ^{10}F-hAβ(1−28). Peptides were prepared by diluting peptide stock solutions into 20 mM sodium phosphate buffer at pH 7.3, containing 150 mM NaCl. Peptide (100 mM) containing Zn^{2+} (0−2 mM) were incubated for 24 h and centrifuged at 13000*g* for 10 min. Peptide concentrations, relative to an uncentrifuged control without Zn^{2+}, were determined by fluorescamine assay. All data points are means ± SD, $n = 3$.

To explore the importance of Zn^{2+} concentrations for metal-induced aggregation of Aβ, 100 μM of each peptide was incubated at pH 7.3 with Zn^{2+} at concentrations of 25 μM, 100 μM, 200 μM, 500 μM, 1 mM, and 2 mM. All peptides aggregated in a Zn^{2+} concentration-dependent manner (Figure 6). A significant difference in the degree of aggregation for hAβ(1−28) and rAβ(1−28) can be seen at each concentration. In addition, ^{13}R-hAβ(1−28) has a similar profile to that of rAβ(1−28), while ^{5}G-hAβ(1−28) and ^{10}F-hAβ(1−28) are similar to hAβ(1−28). The results can be quantitated by calculating an EC_{50} value for each peptide, which is defined as the concentration of Zn^{2+} needed for a 50% reduction of soluble peptide (*53*). The EC_{50} values are 150 ± 50 μM for hAβ(1−28), ^{5}G-hAβ(1−28), and ^{10}F-hAβ(1−28) and 850 ± 100 μM for rAβ(1−28) and ^{13}R-hAβ(1−28). These results again clearly implicate histidine-13 as playing an important role in the Zn^{2+}-induced aggregation of Aβ.

Affinity of hAβ(1−28), rAβ(1−28), ^{5}G-hAβ(1−28), ^{10}F-hAβ(1−28), and ^{13}R-hAβ(1−28) for Metal Chelates. Immobilized iminodiacetate−metal chelates (IDA−M^{2+}) have been applied to the chromatography of both rat and human APP (*54*). In addition, both human Aβ(*1−42*) and Aβ(1−28) display affinity for immobilized metal chelates, including Cu^{2+}, Ni^{2+}, and Zn^{2+} (*49*). Using IDA−M^{2+} coupled with HPLC, we explored the affinity of each of the peptides hAβ(1−28), rAβ(1−28), ^{5}G-hAβ(1−28), ^{10}F-hAβ(1−28), and ^{13}R-hAβ(1−28) for Fe^{3+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} metal chelates (Table 1). None of the peptides were retained on the immobilized metal chelate IDA−Fe^{3+}. All peptides were strongly retained on IDA−Cu^{2+} and only eluted at pH 4.0 when the metal was stripped off the column. The peptides had intermediate affinity for IDA−Zn^{2+} and IDA−Ni^{2+}, with a significant difference in affinity being observed among peptides with both metal chelates. The difference for IDA−Zn^{2+} was the most pronounced, with hAβ(1−28), ^{5}G-hAβ(1−28), and ^{10}F-hAβ(1−28) showing strong affinity and eluting near pH 4.0 and rAβ(1−28) and ^{13}R-hAβ(1−28) showing no affinity and eluting near pH 7.3. For IDA−Ni^{2+}, ^{13}R-hAβ(1−28) eluted slightly before rAβ(1−28) near pH 5.0, while hAβ(1−28), ^{5}G-hAβ(1−28), and ^{10}F-hAβ(1−28) eluted later near pH 4.0. Because at pH 4.0 the metal is removed from the chelate, it is not possible to distinguish the affinity of binding for peptides that elute at this pH.

DISCUSSION

In this study both hAβ(1−28) and rAβ(1−28) showed no β-sheet structure in aqueous solution and did not aggregate significantly in the absence of metals. This is consistent with previous results where the C-terminal amino acids have been shown to be crucial for Aβ aggregation in aqueous solution. hAβ(29−42) has been shown to adopt a β-sheet conformation in aqueous solution independently of pH (*22*−*24*). This C-terminal region contains only hydrophobic amino acids and would be expected to increase both β-sheet and aggregation propensity by increasing hydrophobicity. The side chains within this region would not be expected to be involved in metal coordination due to the absence of charged residues. Metal-induced aggregation of Aβ appears to be initiated by metal coordination with charged residues within the amino acid region 6−28 of Aβ (*38*). In this study we have shown that histidine-13 is involved in Zn^{2+}-induced aggregation of Aβ and is probably involved directly in Zn^{2+} coordination. In agreement with some reports (*28*), but not others (*55*), we observed that the Zn^{2+}-induced aggregation of human Aβ is more rapid and extensive than that of rat Aβ. This difference can be explained by the substitution of histidine-13 for arginine in the rat peptide.

Iodination of Aβ at tyrosine-10 has been reported to decrease the susceptibility of this peptide to Zn^{2+}-induced aggregation (*28*). Because this residue is substituted with a phenylalanine in rat Aβ, it was suggested that tyrosine is critical in coordinating Zn^{2+} to the human peptides. However, we find that ^{10}F-hAβ(1−28) is as susceptible to Zn^{2+}-induced aggregation as is hAβ(1−28), and only ^{13}R-Aβ(1−28) has a lower susceptibility similar to that of rAβ(1−28). These results indicate that tyrosine is not directly involved in the coordination of Zn^{2+} with Aβ. The decreased susceptibility

previously observed for iodinated Aβ could be due to the large iodine atom either interfering sterically with the close β-sheet packing of Aβ or sterically inhibiting zinc binding to a nearby coordination site.

It has been reported that Cu^{2+}, Ni^{2+}, and Zn^{2+} will each displace radiolabeled $^{65}Zn^{2+}$ from Aβ, while Fe^{2+} will not (*41*). Using immobilized iminodiacetate−metal chelation chromatography, we have shown that hAβ(1−28) is strongly retained by IDA−Cu^{2+}, IDA−Zn^{2+}, and IDA−Ni^{2+}, while rAβ(1−28) is strongly retained by IDA−Cu^{2+}, weakly retained by IDA−Ni^{2+}, and not retained by IDA−Zn^{2+}. Neither hAβ(1−28) nor rAβ(1−28) was retained by IDA−Fe^{3+}. Results obtained for ^{13}R-Aβ(1−28) were similar to those for rAβ(1−28), while those for ^{5}G-hAβ(1−28) and ^{10}F-hAβ(1−28) were similar to those for hAβ(1−28) (Table 1). These results indicate that histidine-13 is involved in the Aβ coordination of both zinc and nickel ions and are consistent with these metals binding at the same binding site. The results for IDA−Cu^{2+} may just show that Aβ binding to this metal is particularly strong and does not mean that the Cu^{2+} binding site is different from that of Zn^{2+}. In addition, because two coordination sites are used for metal chelation to IDA, affinity for IDA−metal chelates is not completely analogous to metal binding in solution, which may explain why Fe^{3+}-induced partial aggregation of both hAβ(1−28) and rAβ(1−28) (Figure 4) even though neither of these peptides was retained by IDA−Fe^{3+}.

Although a number of groups have shown that Zn^{2+} will induce the aggregation of Aβ, there is some controversy as to the concentrations of Zn^{2+} required to induce aggregation and the difference in response between rat and human Aβ (*28*, *30*, *55*). Although we observe a significant difference in Zn^{2+}-induced aggregation between hAβ(1−28) and rAβ-(1−28), both peptides will only aggregate at high concentrations of both metal and peptide. The high concentration of peptide needed is not surprising in light of the high aqueous solubility and low β-sheet propensities of the Aβ(1−28) relative to full-length Aβ. However, metal binding affinity should be similar for Aβ(1−28) and full-length Aβ if binding to the monomeric 6−28 sequence is the sole contributor to binding affinity. The EC_{50} of 150 ± 50 μM obtained for hAβ(1−28) is consistent with the EC_{50} of 120−140 μM obtained for the Zn^{2+}-induced aggregation of endogenous Aβ (*53*) and the similar micromolar levels observed by other groups (*30*, *56*). A possible explanation for the nanomolar binding levels reported by Bush et al. (*28*) for full-length Aβ is that a second binding mode dependent on secondary structure may exist. That is, Zn^{2+} may also bind to the aggregated β-sheet structure, in a similar mode to the binding of Cu^{2+} to the β-sheet regions of galactose oxidase and superoxide dismutase (*57*). This high-affinity binding site may not be present in the hAβ(1−28), which forms β-structure less avidly than full-length Aβ. Further work is required to determine if such a structure-dependent high-affinity binding site exists for Aβ. In addition, because the EC_{50} value obtained in this study is a measure of Zn^{2+} required for aggregation rather than a direct measure of Zn^{2+} binding affinity, the lower aggregation propensity of hAβ-(1−28) in the absence of Zn^{2+} could significantly raise the EC_{50} in the presence of Zn^{2+} relative to full-length Aβ.

Although we have shown that histidine-13 is crucial to Zn^{2+}-induced aggregation of Aβ, most probably due to the histidine-13 side chain being a coordination site for Zn^{2+}, the full mechanism of Aβ binding to Zn^{2+} remains unknown. A recent detailed study of a zinc metalloenzyme, carbonic anhydrase II (*58*), showed the zinc ion was liganded to the imidazole side chains of three histidine residues, with a carboxyl group from another spatially close residue coordinate with Zn^{2+} as the fourth ligand, to complete a tetrahedral metal coordination polyhedron. It is expected that Zn^{2+} would coordinate in a similar tetrahedral arrangement with one or more Aβ molecules. The importance of histidine-13 also implicates the adjacent histidine-14 in the coordination of Zn^{2+}. If each zinc ion coordinates with one Aβ peptide, then one could envisage coordination to the three histidine side chains at positions 6, 13, and 14, and a carboxyl group from a glutamic acid or aspartic acid residue. Such an arrangement could potentially stabilize β-sheet formation and thus aggregation. The recent solid-state NMR study by Benzinger et al. (*59*) predicts a parallel rather than an antiparallel β-sheet arrangement for Aβ amyloid. All the parallel β-sheet arrangements that were consistent with the NMR data placed histidine-13 and histidine-14 of each strand within coordinating distance of the equivalent histidine residues in an adjacent strand. In such a parallel arrangement it is possible that each zinc ion is coordinated to four histidine residues on two Aβ strands, stabilizing intermolecular β-sheet structure and providing a seed for amyloid formation. Further work is needed to fully define the binding site of Zn^{2+} and other divalent metals.

The importance of histidine-13 for metal-induced aggregation of Aβ, which may be an important mechanism of amyloid formation in vivo, provides potentially useful information toward the mechanism-based design of aggregation inhibitors. A therapeutic strategy can be envisaged where inhibitors are designed to bind to the amino acid sequence near residue 13 and inhibit metal coordination to this residue.

ACKNOWLEDGMENT

We thank Bill Sawyer for help with sedimentation experiments and Weilan He for help with some CD experiments. We also thank Kevin Barnham, Ashley Bush, Andrew Clippingdale, Andrew Thompson, Michael Jobling, Roberto Cappai, Robert Cherny, and Colin Masters for helpful discussion.

REFERENCES

1. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, C. G., McDonald, B. L., and Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4245−4249.
2. Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K., and Muller-Hill, B. (1987) *Nature 325*, 733−736.
3. Selkoe, D. J. (1993) *Trends Neurosci. 16*, 403−406.
4. Mullan, M., and Crawford, F. (1993) *Trends Neurosci. 16*, 398−403.
5. Yankner, B. A., Duffy, L. K., and Kirschner, D. A. (1990) *Science 250*, 279−282.
6. Roher, A. E., Ball, M. J., Bhave, S. V., and Wakade, A. R. (1991) *Biochem. Biophys. Res. Commun. 174*, 572−579.
7. Pike, G. J., Walencewicz, A. J., Glabe, C. G., and Cotman, C. W. (1991) *Brain Res. 563*, 311−314.
8. Pike, C. J., Burdick, D., Walencewicz, A. J., Glabe, C. G., and Cotman, C. W. (1993) *J. Neurosci. 13*, 1676−1687.
9. Yankner, B. A. (1992) *Neurobiol. Aging 13*, 615−616.

10. Walsh, D. M., Lomakin, A., Benedek, G. B., Condron, M. M., and Teplow, D. B. (1997) *J. Biol. Chem. 272*, 22364−22372.
11. Harper, J. D., Wong, S. S., Lieber, C. M., and Lansbury, P. T., Jr. (1997) *Chem. Biol. 4*, 119−125.
12. Glenner, G. G., and Wong, C. W. (1984) *Biochem. Biophys. Res. Commun. 120*, 885−890.
13. Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. E., Rozovsky, I., Trommer, B., Viola, K. L., Wals, P., Zhang, C., Finch, C. E., and Krafft, G. A. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 6448−6453.
14. Kuo, Y.-M., Webster, S., Emmerling, M. R., De Lima, N., and Roher, A. E. (1998) *Biochim. Biophys. Acta 1406*, 291−298.
15. Strittmatter, W. J., Saunders, A. M., Schenechel, D., Pericak-Vance, M., Egnhild, J., Slavensen, G. S., and Roses, A. D. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8098−8102.
16. Perry, G. Siedlak, S. Mulvihill, P., Kancherla, M., Mijares, M., Kawari, M., Gambetti, P., Sharma, S., Maggiora, L., and Cornette, J. (1989) *Prog. Clin. Biol. Res. 317*, 1021−1025.
17. Snow, A. D., Mar, H., Noclin, D., Sekiguchi, R. T., Kimata, K., Kioke, Y., and Wight, T. N. (1990) *Am. J. Pathol. 137*, 1253−1270.
18. LaDu, M. J., Falduto, M. T., Manelli, A. M., Reardon, C. A., Getz, G. S., and Frail, D. E. (1994) *J. Biol. Chem. 269*, 23403−23406.
19. Wood, S. J., Chan, W., and Wetzel, R. (1996) *Biochemistry 35*, 12623−12628.
20. Wisniewski, T., and Frangione, B. (1992) *Neurosci. Lett. 135*, 235−238.
21. Castano, E. M., Prelli, R., Wisniewski, T., Golabrek, A., Kumar, R. A., Soto, C., and Frangione, D. (1995) *Biochem. J. 306*, 599−604.
22. Barrow, C. J., and Zagorski, M. G. (1991) *Science 253*, 179−182.
23. Barrow, C. J., Yasuda, A., Kenny, P., and Zagorski, M. G. (1992) *J. Mol. Biol. 225*, 1075−1093.
24. Zagorski, M. G., and Barrow, C. J. (1992) *Biochemistry 31*, 5621−5631.
25. Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C., and Glabe, C. (1992) *J. Biol. Chem. 267*, 546−554.
26. Hilbrich, C., Kisters-Woike, B., Reed, J., Masters, C. L., and Beyreuther, K. (1991) *J. Mol. Biol. 218*, 149−163.
27. Vitek, M. P., Bhattacharya, K., Glendening, J. M., Stopa, E., Vlassara, H., Bucala, R., Manogue, K., and Cerami, A. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 4766−4770.
28. Bush, A. I., Pettingell, W. H., Multhaup, G., Paradis, M., Vonsattel, J. P., Gussella, J. F., Beyreuther, K., Masters, C. L., and Tanzi, R. E. (1994) *Science 265*, 1464−1467.
29. Atwood, C. S., Moir, R. D., Haung, X., Scarpa, R. C., Becarra, N. M. E., Romano, D. M., Harshorn, M. A., Tanzi, R. E., and Bush, A. I. (1998) *J. Biol. Chem. 273*, 12817−12826.
30. Mantyh, R. W., Ghilardi, J. R., Rogers, S., DeMaster, E., Atlen, C. J., Stimson, E. R., and Maggio, J. E.. (1993) *J. Neurochem. 61*, 1171−1174.
31. Huang, X., Atwood, C. S., Moir, R. D., Hartshorn, M. A., Vonsattel, J.-P., Tanzi, R. E., and Bush, A. I. (1997) *J. Biol. Chem. 272*, 26464−26470.
32. Huang X., Atwood, C. S., Goldstein, L. E., Hartshorn, M. A., Moir, R. D., Multhaup, G., Tanzi, R. E.; Bush A. I. (1997) *Soc. Neurosci. Abstr. 23*, 1663.
33. Danscher, G., Howell, G. A., Perez-Clausell, J., and Hertel, N. (1985) *Histochemistry 83*, 419−422.
34. Assaf, S. Y., and Chung, S. H. (1984) *Nature 308*, 734−736.
35. Howell, G. A., Welch, M., and Frederickson, C. J. (1984) *Nature 308*, 736−738.
36. Koh, J. Y., Suh, S. W., Gwag, B. J., He, Y. Y., Hsu, C. Y., and Choi, D. W. (1996) *Science 272*, 1013−1016.
37. Wakita, H., Tomimoto, H., Akiguchi, I., Ohnishi, K., Nakamura, S., and Kimura, J. (1992) *Neurosci. Lett. 146*, 135−138.
38. Bush, A. I., Pettingell, W. H., Jr., Paradis, M. D., and Tanzi, R. E. (1994) *J. Biol. Chem. 269*, 12152−12158.
39. Johnstone, E. M., Chaney, M. O., Norris, F. H., Pascual, R., and Little, S. P. (1991) *Mol. Brain Res. 10*, 299−305.
40. Shivers, B. D., Hilbich, C. Multhaup, G., Salbaum, M,. Beyreuther, K., and Seeburg, P. H. (1988) *EMBO J. 7*, 1365−1370.
41. Clements, A., Allsop, D., Walsh, D. M., and Williams, C. H. (1996) *J. Neurochem. 66*, 740−747.
42. Kirschner, D. A., Inouye, H., Duffy, L. K., Sinclair, A., Lind, M., and Selkoe, D. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6953−6957.
43. Gorevic, P. D., Castano, E. M., Sarma, R., and Frangione, B. (1987) *Biochem. Biophys. Res. Commun. 147*, 854−863.
44. Alewood, D., Schnolzer, M., Alewood, P., Jones, A., and Kent, S. B. (1992) *Int. J. Pept. Protein Res. 40*, 180−193.
45. Kaiser, E., Colescott, R. L., Bossinger, C. D., and Cook, P. I. (1970) *Anal. Biochem. 34*, 595−598.
46. Pike, C. J., Walencewicz-Wasserman, A. J., Kosmoski, J., Cribbs, D. H., Glabe, C. G., and Cotman, C. W. (1995) *J. Neurochem. 64*, 253−265.
47. Pike, C. J., Overman, M. J., and Cotman, C. W. (1995) *J. Biol. Chem. 270*, 23895−23898.
48. Arnold, F. H. (1991) *Bio/Technology 9*, 151−156.
49. Balakrishnan, R., Parthasarathy, R., and Sulkowski, E. (1998) *J. Pept. Res. 51*, 91−95.
50. Bˆhlen, P., Stein, S., Dairman, W., and Udenfriend, S. (1973) *Arch. Biochem. Biophys. 156*, 213−220.
51. Luo, P., and Baldwin, R. L. (1997) *Biochemistry 36*, 8413−8421.
52. Morrisett, J. D., David, J. S. K., Pownall, H. J., and Gotto, A. M. (1973) *Biochemistry 12*, 1290−1299.
53. Brown, A. M., Tummola, D. M., Rhodes, K. J., Hofmann, J. R., Jacobsen, J. S., and Sonnenberg-Reines, J. (1997) *J. Neurochem. 69*, 1204−1212.
54. Hesse, L., Beher, D., Masters, C. L., and Multhaup, G. (1994) *FEBS Lett. 349*, 109−116.
55. Esler, W. P., Stimson, E. R., Jennings. J. M., Ghilardi, J. R., Mantyh, P. W., and Maggio, J. E. (1996) *J. Neurochem. 66*, 723−732.
56. Maggio, J. E., Esler, W. P., Stimson, E., Jennings, J. M., Ghilardi, J. R., and Mantyh, P. W. (1995) *Science 268*, 1920−1921.
57. Frausto da Silva, J. J. R., and Williams, R. J. P. (1991) *The Biological Chemistry of the Elements*, Clarendon Press, Oxford, England.
58. Hakansson, E., Carlsson, M., Svensson, L. A., and Liljas, A. (1992) *J. Mol. Biol. 227*, 1192−1204.
59. Benzinger, T. L. S., Gregory, D. M., Burkoth, T. S., Miller-Auer, H., Lynn, D. G., Botto, R. E., and Meredith, S. C. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 13407−13412.

BI990205O