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Structural Changes of Region 1–16 of the Alzheimer Disease Amyloid β -Peptide upon Zinc Binding and *in Vitro* Aging^{*[5]}

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Amyloid deposits within the cerebral tissue constitute a characteristic lesion associated with Alzheimer disease. They mainly consist of the amyloid peptide $A\beta$ and display an abnormal content in Zn^{2+} ions, together with many truncated, isomerized, and racemized forms of $A\beta$. The region 1–16 of $A\beta$ can be considered the minimal zinc-binding domain and contains two aspartates subject to protein aging. The influence of zinc binding and protein aging related modifications on the conformation of this region of $A\beta$ is of importance given the potentiality of this domain to constitute a therapeutic target, especially for immunization approaches. In this study, we determined from NMR data the solution structure of the $A\beta$ -(1–16)- Zn^{2+} complex in aqueous solution at pH 6.5. The residues His⁶, His¹³, and His¹⁴ and the Glu¹¹ carboxylate were identified as ligands that tetrahedrally coordinate the $Zn(II)$ cation. *In vitro* aging experiments on $A\beta$ -(1–16) led to the formation of truncated and isomerized species. The major isomer generated, $A\beta$ -(1–16)-L-iso-Asp⁷, displayed a local conformational change in the His⁶-Ser⁸ region but kept a zinc binding propensity via a coordination mode involving L-iso-Asp⁷. These results are discussed here with regard to $A\beta$ fibrillogenesis and the potentiality of the region 1–16 of $A\beta$ to be used as a therapeutic target.

Amyloid deposition in senile plaques is one of the main cerebral damages associated with Alzheimer disease (AD).³ The amyloid β -peptide ($A\beta$), the major component of these extracellular deposits, is a 39–43-

amino acid peptide that results from the normal proteolytic processing of the amyloid precursor protein.

Considerable NMR data obtained on $A\beta$ and on fragments argue that the soluble monomeric peptide is in an unordered conformation in aqueous solution and mainly adopts an α -helical structure in membrane-mimicking media (1–9). By contrast, the conformation of $A\beta$ within amyloid fibrils consists mainly of a β -sheet structure (10–12). Therefore, as for other conformational diseases (13), a transconformation toward β -sheet is assumed to trigger the abnormal fibrillar amyloid deposition associated with AD. The N-terminal region of $A\beta$ has been shown to be flexible and accessible within amyloid fibrils (14, 15). Therefore, it constitutes an attractive therapeutic target, as illustrated by the ability of monoclonal antibodies directed toward this region to dissociate amyloid fibrils (16, 17).

Imbalances in transition metal cations such as copper, zinc, and iron are assumed to contribute to $A\beta$ deposition (18). In particular, (i) Zn^{2+} ions have been shown to trigger $A\beta$ aggregation (19–21), and (ii) abnormally high levels of zinc have been found within amyloid deposits in AD patients (22, 23). However, the role of zinc in amyloid deposition is subject to debate (24, 25) as (i) Zn^{2+} ions induce the deposition of $A\beta$ in the form of nonfibrillar aggregates (26, 27), and (ii) zinc has been shown to function as an antioxidant able to protect from extensive redox chemical reactions that contribute to AD-related oxidative stress (28, 29). The obligatory sequence involved in zinc binding has been mapped to the region 6–28 of $A\beta$ (30), and the involvement of the His¹³ and His¹⁴ residues has been strongly suggested (31–33). Because of marked aggregation of $A\beta$ peptides or $A\beta$ fragments in the presence of Zn^{2+} , zinc-induced conformational switching has not yet been characterized. However, using $A\beta$ -(1–16), a synthetic peptide spanning the amino acid sequence between the α - and β -secretase cleavage sites, we showed in our initial CD study that this fragment of $A\beta$ selectively bound Zn^{2+} to form a 1:1 complex soluble in physiologically relevant conditions and subsequently underwent a conformational change (34). We proposed a model of zinc attachment that involved the three histidines, His⁶, His¹³, and His¹⁴, and hypothesized Arg⁵ as the fourth coordination ligand from the analysis of the $A\beta$ -(1–16)- Zn^{2+} 1:1 complex in the gas phase by electrospray-ionization mass spectrometry (ESI-MS) (35). Recently, the zinc binding property of $A\beta$ -(1–16) was studied by Mekmouche *et al.* (36), and the apparent dissociation constant of the 1:1 complex was determined.

In addition to its zinc binding propensity, the 1–16 N-terminal region of $A\beta$ is subject to isomerization and racemization of the aspartate residues, which constitute very common types of aging-related protein

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The atomic coordinates and structure factors (code 1ZE7, 1ZE9, and 2BP4) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (<http://www.rcsb.org/>).

[5] The on-line version of this article (available at <http://www.jbc.org>) contains Fig. S1 and Tables A and B.

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³ The abbreviations used are: AD, Alzheimer disease; ACN, acetonitrile; $A\beta$, amyloid β -peptide; $A\beta$ -(1–16), 1–16 N-terminal region of $A\beta$; CSD, chemical shift deviations; DQF-COSY, double quantum filtered correlation spectroscopy; ESI, electrospray ionization; GC, gas chromatography; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum correlation; MS, mass spectrometry; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; RP-HPLC, reversed phase-high performance liquid chromatography; TOCSY, total correlation spectroscopy.

N-terminal Region 1–16 of A β in Alzheimer Disease

damages. These chemical modifications occur through a common pathway involving a neutral cyclic succinimide intermediate (37, 38). The potential contribution of such aspartyl modifications to A β amyloidosis has been addressed, because unusually high contents of racemized and isomerized Asp residues were found in A β isolated from amyloid deposits (39). In addition, these modifications were shown to be related to an increase in β -sheet content and to *in vitro* fibrillation (40, 41), leading to the protein aging hypothesis of AD (42). On the other hand, the assessment of plaque age by using antibodies targeting specifically a particular isomer of A β suggests that these modifications would occur rather after the amyloid deposition (43).

The N-terminal region of A β appears as an attractive therapeutic target, especially for active or passive immunization approaches. Indeed, only the antibodies raised against the N-terminal part of A β are able to reduce the plaque burden and restore cognitive deficits in the mice model of AD (16, 44, 45). In addition, targeting this region should enable us to exert this effect without eliciting an inflammatory response, which had been critical in the first clinical tests of active immunization on humans (46). We have shown previously that A β -(1–16) zinc binding induces an agonist effect on the 4–10 epitope recognition by different monoclonal antibodies, suggesting a folding of the peptide that would render the epitope more accessible (47). Because clinical testing for passive immunization has started, it is of major importance to characterize the structural changes of the N-terminal region of A β upon zinc binding and protein aging-induced modifications.

Here we used NMR and molecular modeling to determine the three-dimensional structure of the apoA β -(1–16) and the A β -(1–16)-Zn²⁺ complex in aqueous solution at pH 6.5. Furthermore, we investigated the ability of A β -(1–16) to undergo protein aging-related modifications. *In vitro* aging experiments were performed on the synthetic peptide A β -(1–16) in the absence and in the presence of Zn²⁺ ions. The resultant species were isolated and identified, and the main isomers generated were analyzed by NMR. From our study, region 1–16 of A β would behave as an autonomous segment able to undergo both zinc binding-induced structuration and aging-related isomerizations.

EXPERIMENTAL PROCEDURES

Synthetic Peptides—The synthetic peptides used throughout this study were purchased from Eurogentec (Angers, France) with a $\geq 95\%$ purity checked by RP-HPLC and matrix-assisted desorption ionization time-of-flight mass spectrometry (Voyager-DEPro, Applied Biosystems, Courtaboeuf, France). A β -(1–16) (Ac-Asp-Ala-Glu-Phe-Arg⁵-His-Asp-Ser-Gly-Tyr¹⁰-Glu-Val-His-His-Glu¹⁵-Lys-NH₂) was acetylated at the N terminus and amidated at the C terminus; A β -(1–16)^{hemi} was nonacetylated at the N terminus and amidated at the C terminus; A β -(1–16)-L-iso-Asp⁷ was acetylated at the N terminus and amidated at the C terminus and contained one L-iso-Asp residue at position 7.

NMR Spectroscopy—A β -(1–16) was dissolved either in 50 mM sodium phosphate buffer, pH 6.5, or in Tris-*d*₁₁/HCl 22.5 mM, pH 5.8, prepared in H₂O/D₂O (9:1) using MilliQTM water samples (Millipore, Saint-Quentin-en-Yvelines, France). The pH was checked directly in the 5-mm Wilmad NMR tubes by using a Mettler Toledo U402-M3-S7/200 electrode. pH values were uncorrected for deuterium isotope effects. Samples for experiments in the presence of Zn²⁺ were prepared by adding a concentrated stock solution of the highest analytical grade ZnCl₂ (Aldrich) in order to reach a Zn²⁺/peptide ratio of 1.5. The absence of pH variation upon zinc addition was checked. Alternatively, A β -(1–16) and A β -(1–16)-L-iso-Asp⁷ were analyzed at 3 mM in 50 mM sodium phosphate buffer, pH 7.5, prepared in H₂O/D₂O (9:1).

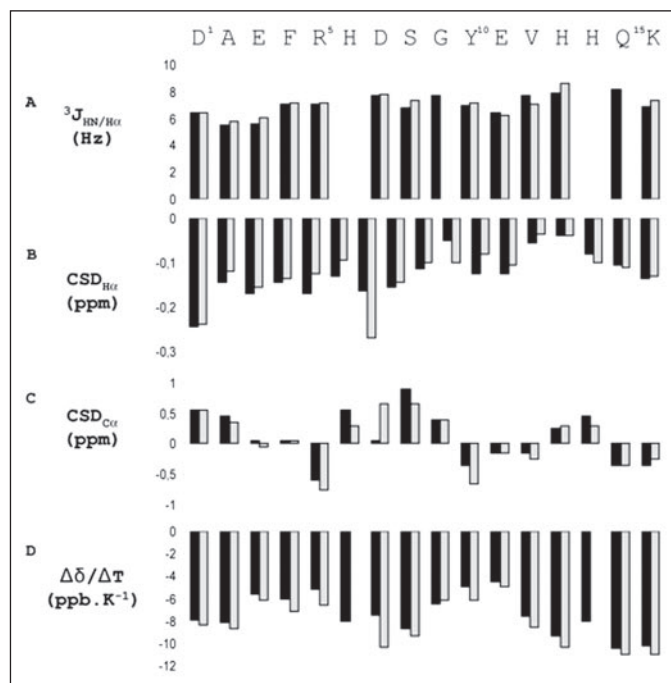


FIGURE 1. Conformational parameters for A β -(1–16) (black) and A β -(1–16)-L-iso-Asp⁷ (gray) at pH 7.4. A, $^3J_{\text{HN-H}\alpha}$ coupling constants. B and C, chemical shift deviations calculated for the H- α and C- α according to Refs. 48 and 49. D, $\Delta\delta/\Delta T$ coefficients determined for the amide protons.

NMR experiments were carried out on AVANCE 400 and DMX 600 spectrometers (Bruker Biospin, Wissembourg, France) both equipped with shielded gradients *z* and set up with ¹H broad band reverse gradient and triple resonance ¹H-¹³C-¹⁵N gradient probe heads, respectively. Temperature was controlled with a BCU-05 refrigeration unit and a BVT 3000 control unit on both spectrometers. ¹H and ¹³C chemical shifts were externally referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate. Conventional one- and two-dimensional experiments, one-dimensional ¹H, ¹H-¹H DQF-COSY, TOCSY (using a spin-lock field produced by an MLEV-17 spin-locking sequence for a spin-lock time of 120 ms), NOESY (with mixing times of 100, 200, 300, and 400 ms), as well as natural abundance ¹H-¹³C HSQC and HMBC (with long-range coupling evolution delays of 70 ms and 90 ms) were performed. Water suppression was achieved by means of either selective low power irradiation for the DQF-COSY experiment or pulsed field gradients in a water suppression by a gradient tailored excitation scheme included in the pulse sequences for both TOCSY and NOESY experiments. Data were processed on Silicon Graphics Indigo 2 XL or O₂ workstations, using XWINNMR 3.1 and AURELIA software (Bruker Biospin, Wissembourg, France).

The determination of temperature coefficients ($\Delta\delta/\Delta T_{\text{HN}}$) was achieved for each sample from five series of ¹H one-dimensional and TOCSY spectra recorded between 288 and 313 K. $^3J_{\text{HN-H}\alpha}$ coupling constants were determined from ¹H one-dimensional and DQF-COSY spectra. The chemical shift deviations (CSD) were calculated for H- α and C- α atoms considering the reference chemical shifts proposed by Wishart *et al.* (48, 49) for each amino acid in random structure.

The p*K*_a values of the histidines were measured in the absence of Zn²⁺ ions by using 3.5 mM A β -(1–16) peptide solutions in 10 mM H₂O/D₂O (9:1) sodium phosphate buffer. The effects of pH on the proton chemical shifts of A β -(1–16) were determined from a series of ¹H one-dimensional, TOCSY, and NOESY spectra recorded at different pH values ranging from 3.0 to 9.5 at 278 K. The pH values were adjusted

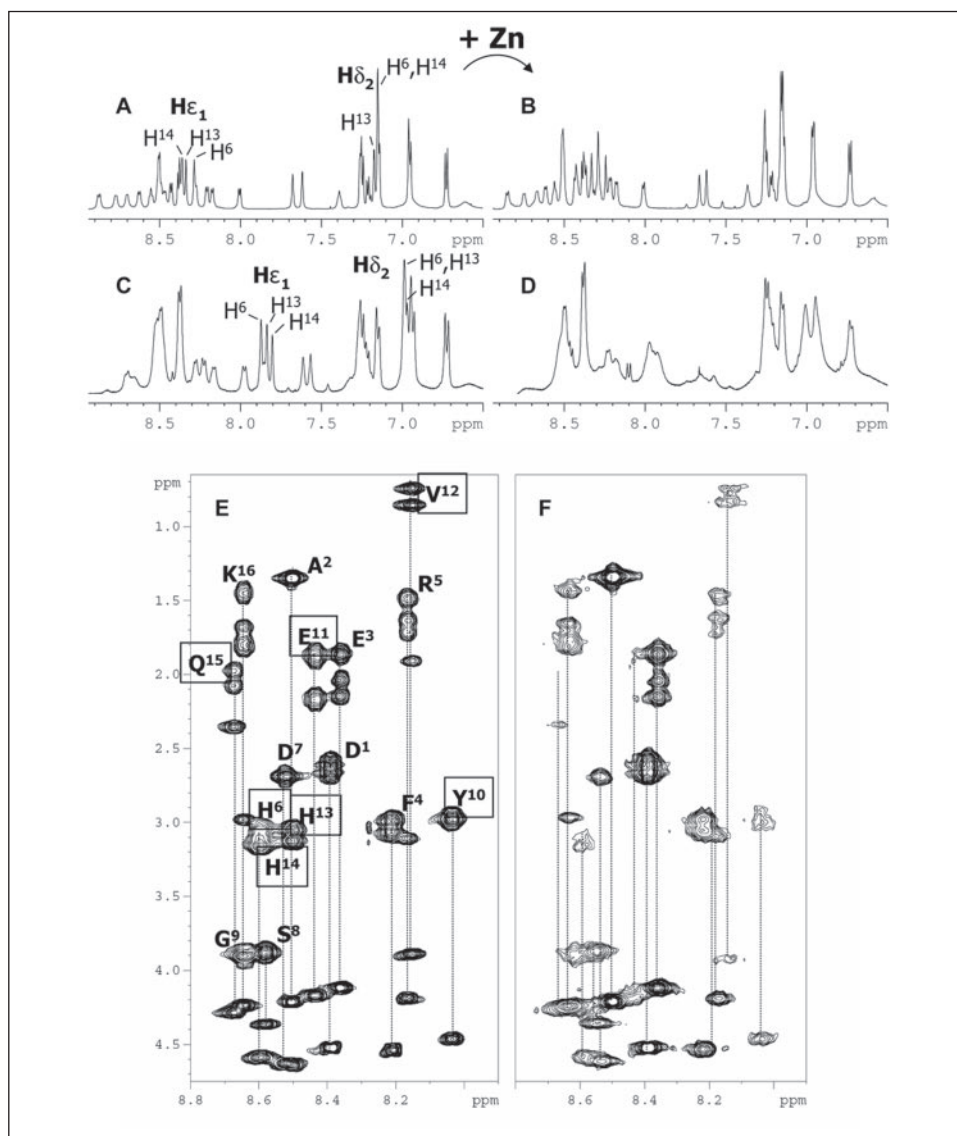


FIGURE 2. ¹H NMR spectra of A β -(1–16) in the absence and in the presence of Zn²⁺ at pH 6.5 and 7.5. A–D, enlargements of the amide and aromatic proton region of the 400-MHz one-dimensional ¹H NMR spectra of 2 mM A β -(1–16). A, pH 6.5; B, pH 6.5, [Zn²⁺] 3 mM; C, pH 7.5; D, pH 7.5, [Zn²⁺] 3 mM. E and F, two-dimensional TOCSY spectra of 3 mM A β -(1–16) at pH 7.5 in the absence (E) and in the presence (F) of 4.8 mM Zn²⁺. Amino acid assignments are provided in E; boxes point those spin systems that undergo the strongest broadening upon Zn²⁺ addition.

before each NMR experiment, and the absence of pH variation over the period of acquisition was checked immediately after. The pK_a values were determined by analyzing the pH titration curves by nonlinear least square fit to the equation $\delta = (\delta_1 + \delta_2 \times 10^{(pH - pK_a)}) / (1 + 10^{(pH - pK_a)})$, where δ is the chemical shift of a resonance measured as a function of pH, and δ_1 and δ_2 are its chemical shifts at the lowest and highest pH values, respectively. This procedure was carried out using the software Curve Expert 1.3. The equation used derives from the Henderson-Hasselbalch equation, assuming a rapid equilibrium between protonated and unprotonated forms (50) and considering a noninteracting model (51).

Distance and Dihedral Angle Constraints—Distance constraints resulting from integrated NOESY spectra and ϕ dihedral angles derived from the $^3J_{\text{HN-H}\alpha}$ coupling constants using the Pardi relation (52) were used for structure calculation. The NOESY experiment with 200 ms of mixing time was selected for distance calculation to get rid of spin diffusion associated with T_1 relaxation. A tolerance range of $\pm 25\%$ of the NMR-derived distances was used to define the upper and lower values of the constraints.

Structure Calculation and Analysis—The three-dimensional structures of A β -(1–16) at pH 6.5 in the absence and in the presence of zinc

were calculated using simulated annealing and energy minimization protocols in X-PLOR 3.851. Alternatively, the conformational calculations were performed with a general purpose internal coordinate molecular dynamics program ICMD (53) by using the variable target function approach (54) adopted for dynamics in the torsion angle space. The program ICMD was further used to calculate the three-dimensional structure of the A β -(1–16)-Zn²⁺ complex. The structures obtained from ICMD calculations are only presented here as final structures.

The molecular dynamics calculations in X-PLOR 3.851 were performed with a target function similar to that used by Nilges *et al.* (55) and a force field adapted for NMR structure determination (parallhdg.pro and topallhdg.pro). When no stereospecific assignments could be made for methyl and methylene protons, the constraints were considered with an appropriate treatment in X-PLOR. Several rounds of structure calculation and assignment were performed to resolve ambiguities. Starting from an extended template structure, a set of 100 structures was calculated. A first phase of 400-ps dynamics (time step = 2 fs) at 1000 K was followed by 80-ps slow cooling step to 100 K (time step = 2 fs; temperature step = 20 K). A weak weight of the van der Waals repulsive term was used at high temperature in order to allow a large conformational sampling. Refinement of the structures was achieved by

N-terminal Region 1–16 of A β in Alzheimer Disease

using the conjugate gradient Powell algorithm with 7000 cycles of energy minimization, using the CHARMM 22 force field (files topallh22x.pro and parallh22x.pro) (56).

The ICMD program was used with the standard geometry of amino acids and peptide bonds and involved multiple cycles of simulated annealing starting from an arbitrary extended peptide conformation. The AMBER99 all-atom force field parameters (57) were applied, with nonbonded interactions truncated at 6 Å by a force-shift method to maintain reasonable atom-atom distances and avoid any bias from longer range interactions. The A β -(1–16) peptide was modeled with all torsion degrees of freedom. According to the variable target function principle (54), all NOE-based distance constraints were first checked for the number of free torsions that separate every particular proton pair. The corresponding number is further referred to as torsion separation. The simulated annealing started from an arbitrary extended conformation obtained by an unrestrained MD simulation under high temperature (7000 K). At the beginning, only constraints of torsion separation one were applied. The following separation levels were added one by one in the course of the protocol when reasonable convergence was achieved for all previous torsion separation levels. As the ICMD protocol does not use pseudoatoms, instead all candidate proton pairs corresponding to a given resonance were analyzed from time to time, and the corresponding constraint was reassigned to the pair with the shortest distance in the current conformation.⁴ The structure calculation of the A β -(1–16)-Zn²⁺ 1:1 complex was carried out using a strategy including two independent steps. In the first step, the structure of the polypeptide chain only was refined in calculations performed without explicit Zn²⁺ ion and without any assumption on zinc coordination. The second calculation step was used in order to obtain a specific peptide-zinc complex structure with a metal coordination geometry satisfying chemical requirements. The experimental set of constraints used in the first step was updated with additional distance and angle constraints that enforced a tetrahedral ligand coordination of the Zn²⁺ ion. To this end, we added four ambiguous constraints that linked the Zn²⁺ ion with all possible partners. These constraints were arbitrarily assigned the torsion separation one, and they were treated along with other constraints, as explained above. Their geometry was derived from zinc-binding sites of relevant high resolution x-ray structures available in the Protein Data Bank. The following atoms and groups present in the A β -(1–16) peptide were considered as potential zinc chelators: N- δ and/or N- ϵ atoms of His⁶, His¹³, and His¹⁴, O- δ 1–O- δ 2 atoms of Asp¹ and Asp⁷, and O- ϵ 1–O- ϵ 2 atoms of Glu³ and Glu¹¹. These Zn²⁺ ligand hypotheses were in agreement with those authorized by the NMR data obtained. The 2.0 Å harmonic distance constraints were applied for the distance between the Zn²⁺ ion and each of its four chelators. If one of the histidine nitrogens took part in the complex, the Zn²⁺ ion was kept in the plane of the imidazole ring and in the bisector plane of the corresponding nitrogen atom by using additional angle constraints. In this case the other nitrogen of the same imidazole ring was temporarily excluded from the list of possible chelators. In a similar way, an appropriate symmetrical orientation of Zn²⁺ with respect to the Asp and Glu carboxyl groups was ensured. The calculated conformers were analyzed using MOLMOL (58), RASMOL (59), and PROCHECK (60) programs. Their coordinates are deposited in the Protein Data Bank.

Production of A β -(1–16) Modified Forms by *in Vitro* Aging—A β -(1–16) (50 μ M or 1.50 mM) or A β -(1–16)^{hemi} (50 μ M) was incubated as described previously (38) in 50 mM Tris-HCl, pH 7.4, containing 0.05% NaN₃ (w/v) either at 37 or 70 °C in the absence or presence of 1 mM

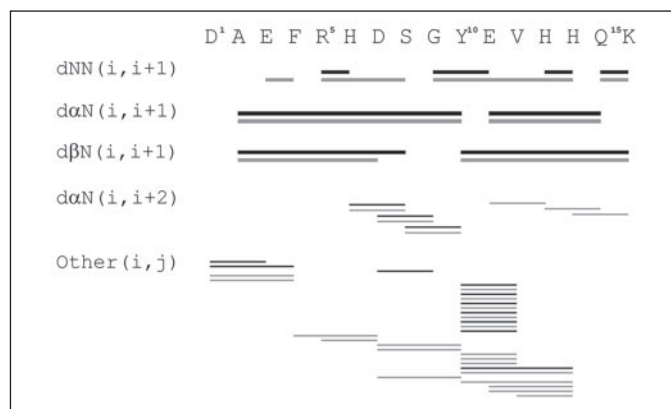


FIGURE 3. Main sequential and medium range NOEs observed for A β -(1–16) at pH 6.5 in the absence (black) and in the presence (gray) of Zn²⁺. The thickness of the bars illustrates the relative cross-peak intensities.

EDTA. The *in vitro* aging experiments in the presence of zinc were conducted with the same protocol in the absence of EDTA and in the presence of ZnCl₂ with a 1:5 peptide/zinc ratio. Reactions were stopped by freezing the samples at –20 °C. The modified peptide mixtures obtained were analyzed at different incubation times by RP-HPLC on a Shiseido C-18 Capcell Pack, 5 μ m, 4.6 \times 250-mm column from Interchim (Montluçon, France), using a linear gradient of 10–40% MeOH in 0.1% aqueous trifluoroacetic acid, at a flow-rate of 1 ml/min. Elution was monitored by measuring the absorbance at 226 nm. Molecular masses of the separated species were determined by liquid chromatography-MS using a Discovery[®]HS C-18, 3 μ m, 2.1 \times 150-mm analytical column (Supelco, St Quentin-Fallavier, France) coupled to an ESI-hybrid quadrupole time-of-flight mass spectrometer (Q-STAR Pulsar, Applied Biosystems, Courtaboeuf, France). The separation was obtained with a linear gradient of 27–35% MeOH in 0.1% aqueous trifluoroacetic acid over 40 min at a flow rate of 200 μ l/min. The same aging protocol was used for the nonacetylated peptide A β -(1–16)^{hemi}. The modified peptide mixtures resulting from the incubation were analyzed at different incubation times by RP-HPLC, as described above, using a linear gradient of 13–18% ACN in 0.1% aqueous trifluoroacetic acid over 40 min at a flow rate of 1 ml/min.

Isolation of A β -(1–16) Isomers Produced from *in Vitro* Aging—The modified peptides resulting from incubation of A β -(1–16) at 70 °C for 14 days were purified by semi-preparative RP-HPLC on a C-18 Uptisphere, 5 μ m, 7.8 \times 250-mm column (Interchim, Montluçon, France) with a linear gradient of 27–35% MeOH in 0.1% aqueous trifluoroacetic acid over 40 min at a flow-rate of 2 ml/min. Absorbance was monitored at 226 nm. Purity of the isolated species was checked by RP-HPLC and ESI-MS (Q-STAR Pulsar, Applied Biosystems, Courtaboeuf, France).

Identification of A β -(1–16) Isomers Produced from *in Vitro* Aging—Truncated peptides were identified by ESI-MS in positive mode (Q-STAR Pulsar, Applied Biosystems, Courtaboeuf, France). The peptide sequences were checked by collision-induced dissociation experiments. Isoaspartic acid residues within A β -(1–16) isomers were quantified by enzymatic methylation catalyzed by the L-isoaspartyl methyltransferase, using the Isoquant[®] protein deamidation kit (Promega, Charbonnières, France), as described previously (61). Briefly, 10 μ l of a 7.5 μ M peptide solution in water were added to the reaction buffer (NaH₂PO₄/Na₂HPO₄ 125 mM, pH 6.8, 1.25 mM EGTA, 0.005% NaN₃, 0.2% Triton X-100) containing L-isoaspartyl methyltransferase and 0.1 mM S-adenosyl-L-methionine. Enzymatic reactions were performed in duplicate at 30 °C for 30 min and stopped by adding 10 μ l of 0.3 M phosphoric acid. Solutions were centrifuged at 10,000 \times g for 8

⁴ T. Malliavin *et al.*, manuscript in preparation.

TABLE 1

Structural statistics of the refined NMR structures of A β -(1–16) in different environments

Each presented NMR structure was calculated using the ICMOD program and corresponds to the group of 20 refined conformers.

Environments	PB ^a	PB-Zn ^b	
		Protocol 1 ^c	Protocol 2 ^c
Target function (\AA^2)	1.60 \pm 0.36	3.10 \pm 0.80	3.30 \pm 0.40
Zinc tetrahedral coordination constraints			
Total			17
Upper distance constraints			4
Planar angle constraints			13
NMR upper distance constraints			
Nonambiguous			
Total	179	253	253
Intra-residue	117	145	145
Sequential	50	75	75
Medium range ($i - j < 5$)	12	29	29
Constraints per residue	11.2	15.8	15.8
Ambiguous			
Total	22	29	29
Constraints per residue	1.4	1.8	1.8
ϕ-torsion angle constraints	5	6	6
Maximum violations			
ϕ -torsion angle constraints ($^\circ$)	0	0	4.4 \pm 1.1
Upper distance constraints (\AA)	0.60 \pm 0.15	0.67 \pm 0.10	0.67 \pm 0.05
No. constraint violations $>0.3 \text{\AA}$	7 \pm 3	7 \pm 3	7 \pm 3
No. constraint violations $>0.2 \text{\AA}$	14 \pm 3	17 \pm 3	17 \pm 3
Ramachandran plot analysis			
Residues in most favored regions (%)	85 \pm 8	85 \pm 8	75 \pm 5
Residues in additionally allowed regions (%)	15 \pm 8	15 \pm 8	25 \pm 5
Structural precision (all residues)			
All atom r.m.s.d. ^d (\AA)	4.17	2.46	1.87
Backbone atom ^b r.m.s.d. (\AA)	3.08	1.39	0.92
Structural precision (residues 4–15)			
All atom r.m.s.d. (\AA)	3.28	1.65	1.14
Backbone N, C- α , C' atom r.m.s.d. (\AA)	1.84	0.72	0.27
Structural precision (residues 4–8)			
All atom r.m.s.d. (\AA)	2.71	1.40	1.30
Backbone atom ^c r.m.s.d. (\AA)	1.19	0.39	0.22
Structural precision (residues 9–15)			
All atom r.m.s.d. (\AA)	1.00	1.14	0.70
Backbone atom ^c r.m.s.d. (\AA)	0.29	0.36	0.14

^a PB indicates phosphate buffer, pH 6.5.^b PB-Zn indicates phosphate buffer, pH 6.5, in the presence of Zn²⁺ with a 1:1.5 A β (1–16)/Zn²⁺ ratio.^c Protocol 1 indicates calculations without any constraints related to zinc binding; protocol 2 indicates calculations using in addition the distance and planar angle constraints described under "Materials and Methods" intended to enforce the tetrahedral coordination of one zinc cation in the A β -(1–16)-Zn²⁺ complex.^d r.m.s.d. indicates root mean square deviation.

min at 4 °C. Supernatants were kept at 4 °C until RP-HPLC analyses, which were performed within the day using a C-18 Uptisphere, 5 μ m, 4.6 \times 250-mm column (Interchim, Montluçon, France) eluted with a linear gradient of 10–30% MeOH in NaH₂PO₄/Na₂HPO₄ 10 mM, pH 6.2, over 20 min at a flow-rate of 1 ml/min. Absorbance was monitored at 260 nm. Iso-Asp residues within the incubation products were quantified by plotting the *S*-adenosylhomocysteine peak intensity on a standard curve drawn up from RP-HPLC profiles of different *S*-adenosylhomocysteine dilutions.

Before N-terminal sequencing, partial hydrolysis of the A β -(1–16) isomers was performed in trifluoroacetic acid at 48 °C (62). The peptides cleaved between residues 1 and 2 were purified by RP-HPLC (Uptisphere, Interchim, 5 μ m, 4.6 \times 250 mm) with a linear gradient of 10–25% ACN in 0.1% aqueous trifluoroacetic acid over 15 min, at a flow rate of 1 ml/min. They were then resuspended in MilliQTM water (Millipore, Saint-Quentin-en-Yvelines, France), and each sample was loaded and argon-dried on a Biobrene-coated filter before being subjected to Edman degradation on a Procise 492 automatic protein sequencer (PerkinElmer Life Sciences).

Absolute configuration of the amino acids of the A β -(1–16) isomers was determined by gas chromatography (GC) analysis of the *N*-triflu-

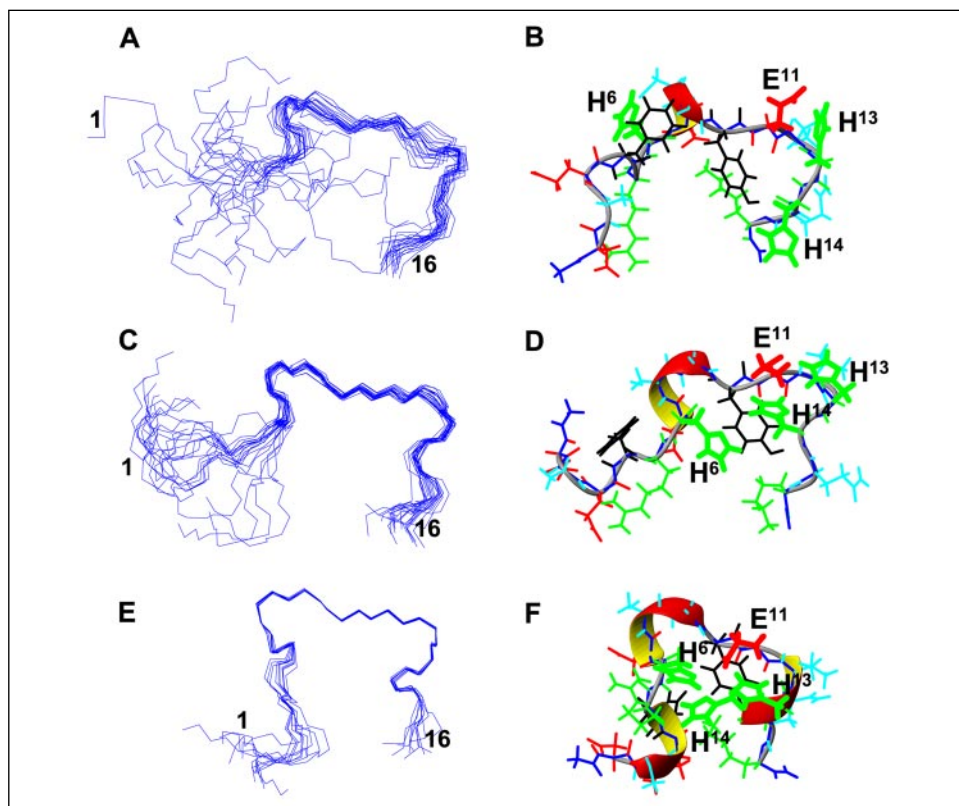
oroacetylisopropyl ester derivatives of the amino acids present in the total hydrolysates, as described previously (63). GC analyses were performed with a 5890 series II chromatograph (Hewlett-Packard, Les Ulis, France) equipped with a flame ionization detector, on a 25-m length, 0.2-mm internal diameter, 0.12- μ m film thickness Chirasil-L-Val (*N*-propionyl-L-valine *tert*-butylamide polysiloxane) quartz capillary column (Varian, Les Ulis, France), using helium as carrier gas at a flow rate of 1.25 ml/min. Injector and detector temperatures were maintained at 300 °C, and the oven temperature was programmed from 50 to 310 °C at a rate of 3 °C/min and then kept at 310 °C for 8 min. The L- or D-configuration of the amino acids was determined by comparing the GC profiles obtained with those of standard L- or D-amino acids derivatized in the same conditions.

RESULTS

Structures of A β -(1–16) and A β -(1–16)-Zn²⁺ in Aqueous Solution—One-dimensional ¹H NMR spectra of A β -(1–16) were recorded over the 0.3–4 mM range of concentrations either in sodium phosphate or Tris-*d*₁₁/HCl buffers at pH 7.4 and 6.5. No differences in line widths or chemical shifts could be detected, supporting the absence of aggrega-

N-terminal Region 1–16 of A β in Alzheimer Disease

FIGURE 4. Three-dimensional ICMD structure of A β -(1–16) at pH 6.5 in the absence (A and B) and in the presence (C–F) of Zn $^{2+}$. The structures in C and D result from calculations performed without any constraints related to the zinc cation, whereas E and F were obtained after introduction of additional constraints relative to the zinc ion coordination. A, C, and E, overlays of the backbone atoms for the 20 selected structures (residues 6–15); B, D, and F, typical structures showing the side chain orientation (backbone in blue, basic residues in green, acidic residues in red, aromatic residues in black, and other residues in cyan). The side chains of His 6 , Glu 11 , His 13 , or His 14 are shown with broader bond lines.



tion of the peptide at such concentrations in agreement with our previous CD data (34).

Spin system identification and complete NMR ^1H assignments (supplemental Table A) were generally obtained from DQF-COSY, TOCSY, and NOESY experiments, using the sequential assignment protocol (64). Spectra recorded at 278 K were primarily used for assignments, and spectra measured at other temperatures, as well as HSQC and HMBC data, were used to resolve ambiguities (see ^{13}C assignments in supplemental Table B).

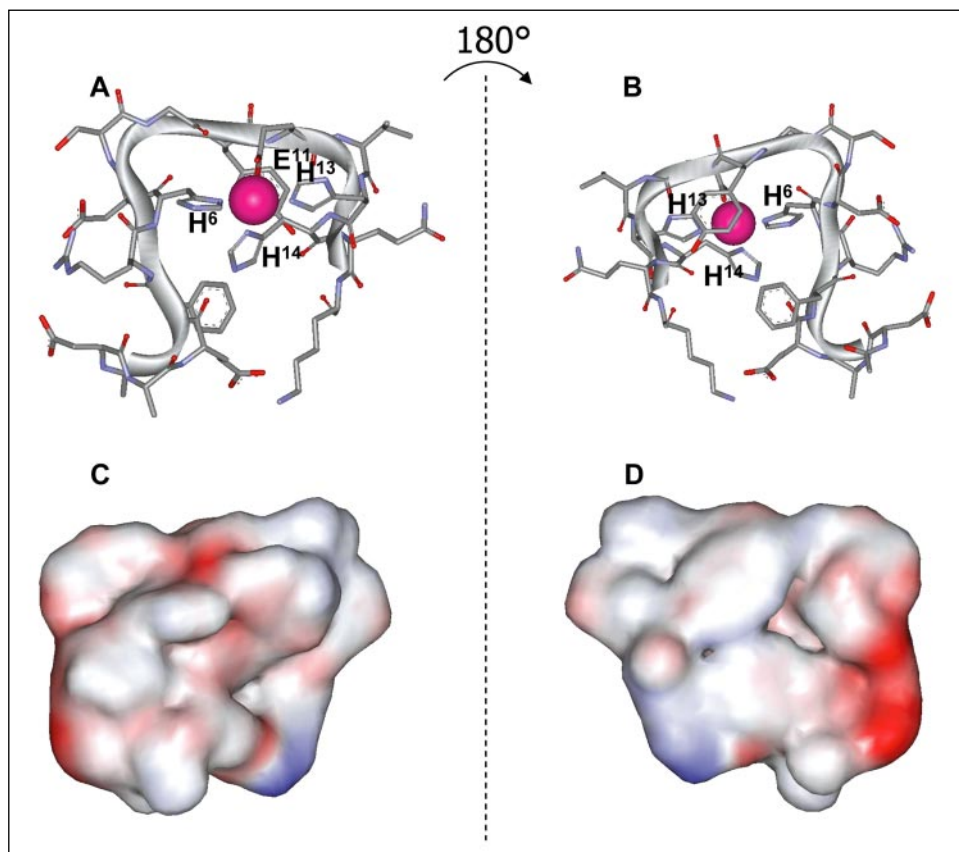
The conformational parameters were analyzed for A β -(1–16) at pH 7.4. They were not indicative of any regular secondary structure elements in the peptide conformation (Fig. 1). However, the $d_{\text{NN}}(i, i + 1)$ and $d_{\alpha\text{N}}(i, i + 2)$ NOEs displayed in the regions 2–5 and 7–13, and in particular the intense d_{NN} between Gly 9 –Tyr 10 , Tyr 10 –Glu 11 , and Glu 11 –Val 12 (data not shown), suggested that these regions folded into a noncanonical structure.

The preliminary analysis of A β -(1–16) by CD showed that it formed a soluble and stable complex with Zn $^{2+}$ ions in the pH 6.0–8.0 range, leading to a conformational change, whereas the homologous peptide with unprotected N and C termini precipitated upon zinc addition (34). Therefore, A β -(1–16) was chosen for a complete NMR analysis in the absence and in the presence of Zn $^{2+}$, in order to characterize the structural change of the N-terminal region 1–16 of A β upon zinc binding. The NMR conformational parameters were acquired at 278 K to ensure observation of the amide protons of His 6 and His 14 , which were not observable at higher temperatures, due to amide proton exchange in the considered pH range. Given the potential contribution of the three histidines to the zinc coordination sphere, their $\text{p}K_a$ values were first measured in order to determine whether these residues were in ionized or neutral form under physiological pH values. The $\text{p}K_a$ values were calculated from nonlinear least square fit of H- $\delta 2$ and H- $\epsilon 1$ proton titration curves to the Henderson-Hasselbalch equation, and the values 7.0, 6.9

and 6.8 ± 0.1 were assigned to His 6 , His 13 , and His 14 , respectively. An equilibrium between protonated and deprotonated imidazole rings was observed in the 6.0–8.0 pH range (supplemental Fig. S1) that corresponded to the pH domain where the zinc-induced conformational change and the soluble A β -(1–16)-Zn $^{2+}$ complex had been previously characterized by CD (34). Addition of Zn $^{2+}$ to a 2 mM A β -(1–16) sample, either in phosphate buffer at pH >6 or in Tris- d_{11} /HCl buffer at pH 5.8 led to the formation of a low amount of precipitate. From our previous analysis of the complex by CD and ESI-MS, this precipitate could be identified as the insoluble fraction of the A β -(1–16)-Zn $^{2+}$ 1:1 complex formed at the higher concentration used for NMR. The supernatant was thus submitted to the NMR experiments. One-dimensional ^1H NMR recordings over the 0.3–3.5 mM range of concentrations discarded the presence of a change in the aggregation state and was in favor of a monomeric state of the 1:1 complex at the 2 mM peptide concentration used for the structure analysis. The ^1H one-dimensional NMR spectra of A β -(1–16) recorded either in the absence or in the presence of 1.5 Zn $^{2+}$ equivalents in sodium phosphate buffer at pH 6.5 and 7.5 did not illustrate any significant Zn $^{2+}$ -induced change in the chemical shifts (Fig. 2, A–D). However, a strong and selective peak broadening was observed at pH 7.5 (Fig. 2, D and F). This mainly concerned region 5–15, whereas the N-terminal region 1–4 remained unaffected. The three histidines were particularly affected, with a complete disappearance of the signals involving the amide protons and H- $\delta 2$ and H- $\epsilon 1$. In addition, the spin systems of Tyr 10 , Glu 11 , and Val 12 nearly disappeared.

Such a peak broadening, similar to that already observed by Curtain *et al.* (28) upon adding Zn $^{2+}$ to a solution of A β -(1–28) could be indicative of exchange between zinc-complexed and uncomplexed A β -(1–16). The absence of any change in the rest of the spectrum was in agreement with the monomeric state of the complex analyzed and indicated that the presence of soluble oligomers that could have resulted in broadened resonances was negligible. Finally, pH 6.5, which allowed good quality

FIGURE 5. Structure of the A β -(1–16)-Zn $^{2+}$ complex at pH 6.5 calculated with the ICMD protocol. A and B, views showing the location of the zinc ion, which is coordinated through the imidazole nitrogens of the three histidine, His 6 , His 13 , and His 14 , side chains and the carboxylate of the Glu 11 side chain. C and D, corresponding electrostatic molecular surface of the complex; C, view is rotated 180° about the x axis relative to the A view. Blue and red correspond to negatively and positively charged areas, respectively.



spectra and was still included in the protonation/deprotonation pH range of the three histidines (supplemental Fig. S1), was selected for investigation of the three-dimensional structure of the apoA β -(1–16) and the A β -(1–16)-Zn $^{2+}$ complex. Although most of the conformational parameters of A β -(1–16), *i.e.* H- α - and C- α -CSD, $^3J_{\text{HN-H}\alpha}$ and $\Delta\delta/\Delta T_{\text{HN}}$, were unchanged upon Zn $^{2+}$ addition, a number of additional specific NOEs were observed in the presence of Zn $^{2+}$ (Fig. 3), which suggested a structural change.

The three-dimensional structures of the apoA β -(1–16) and the A β -(1–16)-Zn $^{2+}$ complex were calculated by NMR-restrained molecular modeling, using the constraints summarized in Table 1. The apoA β -(1–16) appeared poorly structured in the N-terminal 1–6 region (Fig. 4A), whereas an irregular structure was characterized in the 7–15 part, including a turn centered at residues 7–8. The 20 selected structures displayed a good fit of the side chains in the C-terminal region. Their orientation suggested the presence of stabilizing intramolecular hydrogen bonds involving the pairs Asp 7 /Lys 16 and Glu 11 /His 13 , as well as the backbone carbonyl group of His 14 and the Tyr 10 side chain (Fig. 4B). In the presence of Zn $^{2+}$, the modeling of the A β -(1–16) structure using X-PLOR or ICMD programs without introducing any constraints related to the cation binding resulted in a more compact structure (Fig. 4C). Compared with the apoA β -(1–16) folding, the structure of the N-terminal region was better defined, and a global reorientation of most side chains toward the inside of the structure was observed, which particularly concerned those of all three histidines (Fig. 4D). The ICMD protocol (53) was used to assign the peptide/Zn $^{2+}$ -binding sites and obtain the three-dimensional structure of the A β -(1–16)-Zn $^{2+}$ complex (Fig. 4, E and F, and Fig. 5). In most of the selected structures, the Asp 7 -Gly 9 region adopted an irregular 3_{10} helical structure (Fig. 4F). The Zn(II) cation was tetrahedrally coordinated to His 6 , His 13 , and His 14 through their N- δ 1, N- ϵ 2, and N- δ 1 atoms, respectively, and to Glu 11

through its carboxylate. It is worth noting the coordination sphere proposed from our calculation conveniently fits the ^{111}Cd NMR data obtained by Mekmouche *et al.* (36). In all the selected structures, the Phe 4 residue appeared to be located in the inner core of the structure, and Tyr 10 was systematically located on the face opposed to the zinc atom. The binding sites thus obtained corresponded to the more broadened spin systems displayed on the TOCSY spectrum registered in the presence of Zn $^{2+}$ (Fig. 2, D and F). An analysis of the surface features of the complex (Fig. 5, C and D) showed that most of the surface is hydrophobic or neutral, with a negatively charged patch located near the N terminus (Asp 1 and Glu 3) and a single restricted positively charged region due to Lys 16 . This is in agreement with the fact that all positively charged residues present in A β -(1–16) reoriented toward the inner core of the complex.

In Vitro Aging of A β -(1–16) in the Absence and in the Presence of Zn $^{2+}$ Ions and Subsequent Isomerizations and Racemizations—*In vitro* aging of A β -(1–16) was performed by incubation at pH 7.4 and 37 °C, either in the absence or in the presence of Zn $^{2+}$. In order to accelerate the rate of succinimide formation (38) and to facilitate the identification of a maximal range of modified species, another set of experiments was conducted at 70 °C. The aging experiments at 37 °C were conducted over an incubation period of 70 and 130 days in the absence and in the presence of Zn $^{2+}$, respectively (Fig. 6, A and B), whereas this incubation period could be shortened to 14 days at 70 °C. The chromatograms obtained in the absence and in the presence of EDTA were similar (data not shown), ruling out the contribution to the chemical modifications observed of trace metal contaminants, such as copper-induced redox processes.

The Asp 1 - and Asp 7 -isomerized and/or racemized species formed at 70 °C (Fig. 6C) were identified from a panel of experiments, including (i) L-isopartyl methyltransferase-assisted quantification of isoaspartate residues, (ii) Edman sequencing, and (iii) GC analysis on a chiral capil-

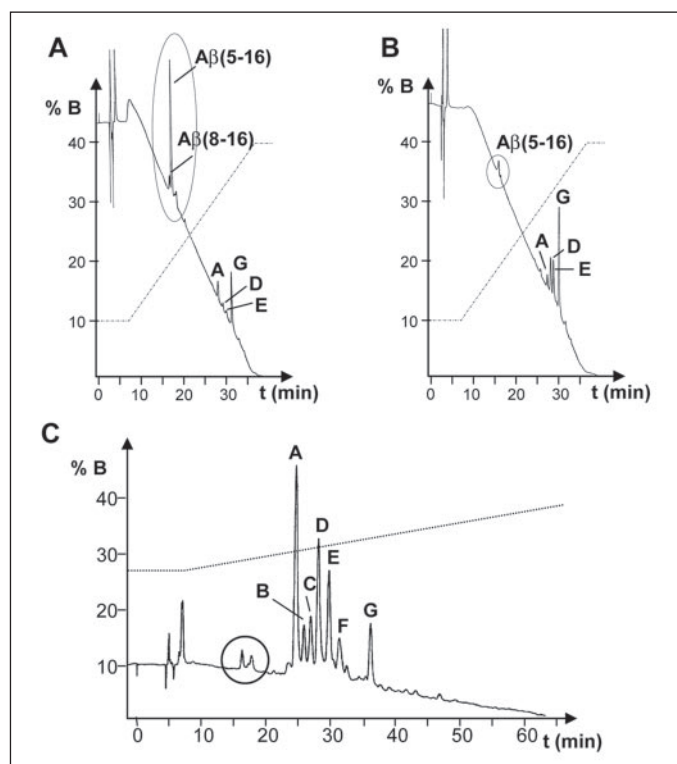


FIGURE 6. RP-HPLC profiles of A β (1–16) obtained upon *in vitro* aging. *A* and *B*, analytical chromatograms obtained after 42 days of incubation at pH 7.4, 37 °C, in the absence of Zn²⁺ (*A*) and after 128 days incubation in the presence of Zn²⁺ (*B*). *C*, semi-preparative chromatogram recorded after 14 days incubation at pH 7.4 and 70 °C. The circled species correspond to truncated forms of A β (1–16). Peaks A–F correspond to the isomers of A β (1–16) (see Table 2), with peak G being unmodified A β (1–16). The MeOH percentages in the gradients used for separations are represented as dotted lines.

lary column of the derivatized amino acids obtained in the total acid hydrolysate (Table 2). The A, D, and E species, which are the three major isomers produced, were assigned to A β (1–16)-L-iso-Asp^{1,7}, A β (1–16)-L-Iso-Asp⁷, and A β (1–16)-L-iso-Asp¹, respectively. Furthermore, liquid chromatography-MS analysis revealed the presence of N- or C-truncated peptides (Fig. 6C, circled peak), which were identified from both their *m/z* ratios and MS/MS data as A β (2–16), A β (1–7), and A β (1–6) (data not shown).

In vitro aging of the nonacetylated A β (1–16)^{hemi} peptide also led to the formation of truncated and isomerized species at 70 °C, but peak overlapping in the HPLC profiles rendered the attribution incomplete (data not shown). The A β (2–16)-, A β (1–7)-, and A β (1–6)-truncated peptides were detected, as for A β (1–16) aging, but the major truncated species formed was A β (3–16). Successful Edman sequencing of this last peptide indicated the absence of the N-terminal pyroglutamate residue in this truncated form.

At 37 °C, the truncated peptides appeared favored as compared with the isomerized species, A β (5–16) being the major modified species from 20 days of incubation (Fig. 6A and 7A). In addition, the racemizations of L-Asp or L-iso-Asp residues to their D-isomers were totally absent. The presence of Zn²⁺ ions in the incubation medium significantly altered the kinetic profile of the *in vitro* aging at 37 °C by disfavoring truncations at the advantage of isoaspartate formation at positions 1 and 7 in the sequence (Fig. 6B and Fig. 7B). By contrast, the presence of Zn²⁺ ions in the incubation medium was without effect on the nature and the relative abundances of the species formed from A β (1–16) at 70 °C (data not shown).

The conformational parameters of A β (1–16)-L-iso-Asp⁷, one of the major species formed in the presence of Zn²⁺, were quite similar to

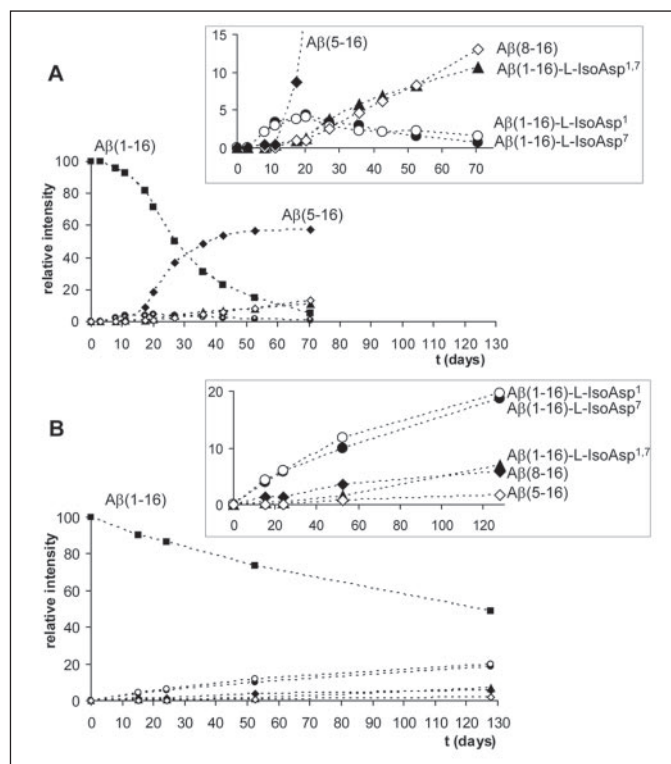


FIGURE 7. Relative intensities of the major species formed upon *in vitro* aging in the absence (*A*) and in the presence (*B*) of Zn²⁺ and detected by RP-HPLC as a function of incubation time. Captions show enlargements of the evolution of the minor species as a function of incubation time.

those of A β (1–16) (Fig. 1). Local changes in the His⁶–Ser⁸ region were observed because of the presence of L-iso-Asp⁷, as illustrated by the CSD profiles (Fig. 1, *B* and *C*). Furthermore, as already observed for L-iso-Asp-containing proteins (65), this residue triggered a change in the intensity of sequential NOEs between residues 7 and 8, with a strengthened d β N connectivity and a weakened α N connectivity (data not shown). However, the observed changes in the NMR parameters appeared to be local and not to express a perturbation of the global peptide fold. One-dimensional ¹H and TOCSY spectra of A β (1–16)-L-iso-Asp⁷ were performed in the presence of Zn²⁺ ions in order to assess the effect of isoaspartate on the A β (1–16)-Zn²⁺ interaction. Because a precipitate formed at pH values above 6, the spectra were recorded at pH 5.8. They showed a particularly strong broadening of the L-iso-Asp⁷ signals, which almost disappeared from the spectra; His⁶ and residues 10–14 were also affected (data not shown). These results indicated that the modified L-iso-Asp⁷ residue did not prevent the interaction and even was contributing to the zinc coordination.

DISCUSSION

The N-terminal 1–16 part of A β is a flexible region that remains accessible in amyloid deposits (14, 15) and is involved in the zinc binding capacity and protein aging propensity of A β . In this study, we characterized for the first time the three-dimensional structure of the soluble A β (1–16)-Zn²⁺ complex as well as the chemical modifications of this region of A β upon protein aging.

Structuration of A β (1–16) Upon Zinc Binding—In phosphate buffer at pH 6.5, the apoA β (1–16) shows a rather well defined structure, particularly in region 7–15, but without any canonical element. The addition of Zn²⁺ to the solution mainly affects the Tyr¹⁰–Gln¹⁵ region and particularly the H- δ 2 and H- ϵ 1 resonances of all three histidines, in agreement with previous data on A β (1–28) (28). The A β (1–16) struc-

TABLE 2

Identification of the isomers of A β -(1–16) produced upon *in vitro* aging at pH 7.4 and 70 °C

The outcome of the L-isoaspartyl methyltransferase (PIMT)-catalyzed methylation, Edman sequencing, and chiral GC results is shown. The asterisk indicates a yield decrease in Edman sequencing.

Isomer	No. (L)-iso-Asp (PIMT)	Sequence Edman	Asp/iso-Asp configuration (GC)	Residues identified	
				Position 1	Position 7
A	2	AEFRH	2 (L)	(L)-iso-Asp	(L)-iso-Asp
B	1	AEFRH	1 (L), 1 (D)	(D)-Asp or (D)-iso-Asp	(L)-iso-Asp
C	1	AEFRH	1 (L), 1 (D)	(D)-Asp or (D)-iso-Asp	(L)-iso-Asp
D	1	AEFRH	2 (L)	(L)-Asp	(L)-iso-Asp
E	1	AEFRHDSGYE...	2 (L)	(L)-iso-Asp	(L)-Asp
F	0	AEFRHD*SGYE...	1 (L), 1 (D)	(L)-Asp	(D)-Asp
G	0	AEFRHDSGYE...	2 (L)	(L)-Asp	(L)-Asp

ture becomes more compact upon zinc binding. The three-dimensional structure of the A β -(1–16)-Zn²⁺ soluble 1:1 complex calculated from the NMR constraints reveals His⁶, Glu¹¹, His¹³, and His¹⁴ as the four ligands involved in the zinc coordination sphere. Such a motif of zinc attachment is reminiscent of a motif identified previously in the case of short peptides designed to bind zinc ions (66). The identification of the three histidines as the zinc ligands does not contradict previous studies using various spectroscopic techniques, which proposed that one to all three histidines in this region of A β acted as metal-binding sites (31–33, 36). The identification of the fourth zinc chelator is much more debated. The present calculation of the complex identifies Glu¹¹ as the fourth partner, acting through its carboxylate side chain. In our previous MS study on the A β -(1–16)-Zn²⁺ complex (35), Arg⁵ was proposed as the additional ligand. This discrepancy with the present work is most likely attributable to the different acidic/basic properties of the amino acids in the gas phase and in solution (67). Tyr¹⁰ has been also suggested previously as a possible ligand participating in Cu²⁺ or Zn²⁺ chelation by A β (28, 32). In the structure obtained here for the A β -(1–16)-Zn²⁺ soluble 1:1 complex, Tyr¹⁰ is excluded from the coordination sphere and is located on the opposite face from the Zn²⁺ ion. This is in agreement with Mekmouche *et al.* (36), who also excluded the involvement of Tyr¹⁰ in the coordination sphere from both NMR and absorption spectroscopy data. In this last study, Asp¹ was proposed as the fourth coordination site of the zinc ion in A β -(1–16) at pH 8.7 (36). This is not consistent with our NMR data at pH 6.5 and 7.5. Those exclude Asp¹ as metal chelator, because (i) the corresponding NMR signals were not broadened upon zinc addition, and (ii) this residue was not selected as contributing to the zinc coordination sphere in our structure calculations, although it was considered as a potential ligand. The lower pH and the N-terminal acetylation of A β -(1–16) in our study can contribute to understanding this difference.

From our results, region 9–15, which contains three of the zinc ligands Glu¹¹, His¹³, His¹⁴, appears as a prefolded region in A β -(1–16). The fourth ligand in the zinc coordination sphere, His⁶, is provided by region 4–8 of A β -(1–16), which is poorly structured. The N-terminal zinc binding region of A β thus behaves as an independent folding unit. Zinc binding to apoA β -(1–16) appears to stabilize the preformed structure of region 9–15 and to induce the folding of the N-terminal region into an irregular 3_{10} helix. The irregular helical structure displayed by most of the structures calculated here for A β -(1–16) suggests that zinc binding to A β promotes its helical rather than β -sheet conformation, as also proposed for longer A β peptides (28, 68). In light of our results, the conformational change of A β -(1–16) in the presence of zinc appears to be rather a stabilization and structuration of a poorly preformed structure than a drastic transconformation. This trend is in agreement with Curtain *et al.* (28) and with our previous observation that a structure stabilization of A β -(1–16) upon Zn²⁺ binding induces a better recognition of this region of A β by monoclonal antibodies (47).

In the absence of zinc, A β -(1–16) displays a bend in its central region, as illustrated by the intense dNN(*i, i + 1*) NOEs between Gly⁹ and Val¹². This bending has also been described for the full-length A β and has been proposed to contribute to A β fibrillation by promoting the formation of salt bridges involving the histidine and aspartic/glutamic acid side chains (69). From our study, zinc binding appears to stabilize this bended structure, which could reinforce the hypothesis of the involvement of zinc in A β aggregation (19–21, 36). However, the nonfibrillar nature of A β aggregates formed upon zinc addition (26, 27) contradicts this interpretation. Furthermore, the involvement of the histidines in zinc binding would rather compete with the formation of salt bridges with aspartic/glutamic acid side chains. Therefore, we propose that zinc binding would not be implicated in A β fibrillogenesis but would rather occur after amyloid deposition as a result of the accessibility of the A β N-terminal region within fibrils. In this context, zinc binding-induced aggregation of A β would result from another mechanism, such as the formation of intermolecular histidine-metal-histidine bridges (18, 28).

Our CD (34) and NMR data have shown that A β -(1–16) is monomeric in the 5 μ M to 1 mM concentration range. The absence of oligomerization upon zinc binding has been shown previously by size exclusion chromatography (36). This trend is also emphasized by the absence of variation in A β -(1–16) NMR chemical shifts and of unspecific NMR signal broadening upon zinc addition. Similarly, Curtain *et al.* (28) have characterized 1:1 monomeric complexes with copper for A β -(1–28) and A β -(1–40). In the conditions of our study, zinc binding to A β -(1–16) appears to lead to the soluble monomeric complex accompanied by a small amount of insoluble aggregates, as proposed by Miura *et al.* (32).

Protein Aging-induced Modifications of A β -(1–16)—Because age-related peptide modifications are suggested to participate in the AD pathogenesis, *in vitro* aging of A β -(1–16) has been investigated here. Numerous modifications of the synthetic peptide are observed, *i.e.* truncations, isomerizations, and racemizations. Truncated peptides, including an N-terminal pyroglutamate at positions 3 and 11 of A β , are species that have been detected within amyloid fibrils (71). Such species are not formed here during the *in vitro* aging process of A β -(1–16) or A β -(1–16)^{hemi} at 37 °C. Thus, the truncated molecular forms detected within fibrils presumably represent by-products of metabolic intermediates toward degradation and are not produced spontaneously upon protein aging.

The incorporation of iso-Asp residues is suggested to increase the tendency to form β -sheet in amyloid peptides (41, 70). Here we observe that the isomerization of L-Asp⁷ to L-iso-Asp leads to a local conformational change in the region His⁶–Ser⁸ but does not affect the overall structure of the peptide. More interestingly, the presence of Zn²⁺ ions significantly changes the chemical modification profile of A β -(1–16) upon the *in vitro* aging process at 37 °C, by favoring isomerizations at the expense of truncations. This trend suggests that the association of

N-terminal Region 1–16 of A β in Alzheimer Disease

the modified peptides A β -(1–16)-L-iso-Asp¹, A β -(1–16)-L-iso-Asp⁷, and A β -(1–16)-L-iso-Asp^{1,7} with Zn²⁺ ions displaces the equilibrium of the A β -(1–16) isomerization reactions toward the formation of L-iso-Asp species. Furthermore, A β -(1–16)-L-iso-Asp⁷, one of the two major modified species generated upon protein aging, appears to bind Zn²⁺ with a different coordination mode, involving the L-iso-Asp⁷ residue itself. Finally, the presence of a peptide bond before Asp¹ does not affect the propensity of this residue to undergo isomerizations/racemizations, because the nonacetylated A β -(1–16)^{hemi} also exhibits aging-related modifications at Asp¹ and Asp⁷.

The impact of the aging-related modifications of the aspartate residues on both the conformation of A β -(1–16) and its zinc binding capacity has been subsequently assessed here. Altogether, our results reflect the high potential of chemical and conformational modifications of region 1–16 of A β upon both aging and zinc binding, which could partly account for its important structural heterogeneity within amyloid fibrils.

Implications for Therapeutic Approaches with Region 1–16 of A β as Target—The occurrence of truncated, isomerized, and racemized species of A β and of zinc-bound species within amyloid deposits must be considered in the frame of a therapeutic approach targeting the N-terminal region of A β . On the one hand, the modified species of A β could be used as immunological targets, while avoiding autoimmune response because of the recognition of the soluble A β . The established potentiality to target specifically a particular isomer of A β present within amyloid deposits (43) suggests the applicability of such an approach to isomerized species of A β . On the other hand, the high zinc content within amyloid plaques might not be adverse but even favorable to the interaction of A β with anti-A β antibodies, which constitutes a positive trend in the frame of an epitope-based vaccination approach (16, 47).

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