Point Substitution in the Central Hydrophobic Cluster of a Human β-Amyloid Congener Disrupts Peptide Folding and Abolishes Plaque Competence†

William P. Esler,‡ Evelyn R. Stimson,‡ Joseph R. Ghilardi,§ Yi-An Lu,|| Arthur M. Felix,|| Harry V. Vinters,⊥ Patrick W. Mantyh,§ Jonathan P. Lee,# and John E. Maggio*,‡

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, Molecular Neurobiology Laboratory (151), Veteran's Administration Medical Center and Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota 55455, Department of Pathology & Laboratory Medicine and Brain Research Institute, University of California at Los Angeles Medical Center, Los Angeles, California 90095-1732, Department of Chemistry, Boston University, Boston, Massachusetts 02215, and Roche Research Center, Hoffmann−La Roche Inc., Nutley, New Jersey 07110

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ABSTRACT: Alzheimer's disease (AD) is pathologically characterized by the presence of numerous insoluble amyloid plaques in the brain composed primarily of a 40−43 amino acid peptide, the human β-amyloid peptide (Aβ). The process of Aβ deposition can be modeled *in vitro* by deposition of physiological concentrations of radiolabeled Aβ onto preexisting amyloid in preparations of unfixed AD cerebral cortex. Using this model system, it has been shown that Aβ deposition is biochemically distinct from Aβ aggregation and occurs readily at physiological Aβ concentrations, but which regions and conformations of Aβ are essential to Aβ deposition is poorly understood. We report here that an active congener, Aβ(10−35)-NH_2, displays time dependence, pH−activity profile, and kinetic order of deposition similar to Aβ(1−40), and is sufficiently soluble for NMR spectroscopy in water under conditions where it actively deposits. To examine the importance of the central hydrophobic cluster of Aβ (LVFFA, residues 17−21) for *in vitro* Aβ deposition, an Aβ(10−35)-NH_2 analog with a single point substitution (F19T) in this region was synthesized and examined. Unlike Aβ(10−35)-NH_2, the F19T analog was plaque growth incompetent, and NMR analysis indicated that the mutant peptide was significantly less folded than wild-type Aβ. These results support previous studies suggesting that the plaque competence of Aβ correlates with peptide folding. Since compounds that alter Aβ folding may reduce amyloid deposition, the central hydrophobic cluster of Aβ will be a tempting target for structure-based drug design when high-resolution structural information becomes available.

Alzheimer's disease (AD)[1] is a progressive neurodegenerative disorder that affects a substantial fraction of the elderly, approximately half of the population over age 85 (Selkoe, 1991, 1994). Like other amyloidoses, AD is pathologically characterized by the presence of numerous insoluble proteinaceous deposits, termed amyloid based on their tinctorial properties. The extracellular amyloid is found both at neuropil sites and in blood vessel walls in the brain and is widely believed to be involved in the progressive neurodegeneration of the disease (Selkoe, 1991, 1994; Mattson et al., 1992; Pike et al., 1991a, 1993, 1995; Kowall et al., 1992). The principal component of these lesions is a hydrophobic 40−43 amino acid peptide (Glenner & Wong, 1984) called β-amyloid peptide (Aβ). Aβ is produced as a cleavage product from a much larger protein termed the amyloid precursor protein (βPP) (Kang et al., 1987; Goldgaber et al., 1987; Tanzi et al., 1987). The peptide is constitutively produced by cells and is present at similar levels ($\approx 10^{-9}$ M) in normal and AD cerebrospinal fluid (CSF) (Haass et al., 1992; Busciglio et al., 1993; Seubert et al., 1992; Shoji et al., 1992; van Gool et al., 1994), indicating that production of the peptide is a normal process rather than a pathological one. The pathogenic role of Aβ in AD (Selkoe, 1991, 1994; Cummings & Cotman, 1995) has been supported by the discovery of several mutations in Aβ or βPP tightly linked to inherited forms of the disease (Mullan & Crawford, 1993; Schellenberg, 1995). Evidence that Aβ plays a causative role in AD pathology has also come from several model systems. Aβ is toxic to neurons in culture (Pike et al., 1991a,b; Busciglio et al., 1993; Roher et al., 1991; Fraser et al., 1994; Yankner et al., 1990), and plaque

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* Correspondence should be addressed to this author at the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115. Telephone: (617) 432-0757. FAX: (617) 432-3833.

‡ Harvard Medical School.

§ University of Minnesota.

|| Hoffmann-La Roche Inc.

⊥ University of California at Los Angeles Medical Center.

Boston University.

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[1] Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer's disease; βPP, β-amyloid precursor protein; BSA, bovine serum albumin; CD, circular dichroism; CSF, cerebrospinal fluid; DMSO, dimethyl sulfoxide; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; ^{125}I-Aβ, ^{125}I-iodotyrosine^{10}-human Aβ(1−40)-OH; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser enhanced spectroscopy; NMR, solution nuclear magnetic resonance spectroscopy; RP-HPLC, reverse-phase high-performance liquid chromatography; Tris, tris(hydroxymethyl)-aminomethane; TSP, sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$; WGS, waveform gradient suppression.

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formation in the brains of transgenic animals overexpressing human Aβ peptide also leads to neuronal toxicity (Games et al., 1995; LaFerla et al., 1995).

The β-amyloid peptide has been shown to self-assemble into filamentous aggregates (Kirschner et al., 1987; Halverson et al., 1990; Hilbich et al., 1991; Burdick et al., 1992; Castaño et al., 1986), and this process has been linked to the toxicity of Aβ observed in cultured neurons (Mattson et al., 1992; Pike et al., 1991a, 1993, 1995; Yankner et al., 1990). As a consequence, much of the work on Aβ has focused on understanding the aggregation properties of the peptide.

Despite significant research efforts over several years, little high-resolution structural information has been obtained about either the soluble monomeric building blocks or the insoluble oligomeric amyloid characterizing AD and other amyloidoses. Many reports have identified either specific residues or segments of Aβ that affect the solubility or toxicity of the peptide in the micromolar to millimolar range (Hilbich et al., 1991, 1992; Burdick et al., 1992; Castaño et al., 1986; Dyrks et al., 1993; Jarrett & Lansbury, 1992, 1993; Jarrett et al., 1993, 1994). However, the concentration of Aβ in human CSF (Seubert et al., 1992; Shoji et al., 1993; van Gool et al., 1994) is much lower (nanomolar and below), and Aβ aggregation[2] *in vitro* does not occur at these levels (Burdick et al., 1992) in the absence of high concentrations of other factors (Mantyh et al., 1993; Esler et al., 1996b). Furthermore, it is not necessarily true that there is a distinct and reliable correlation between *in vitro* solubility and Aβ deposition (Lee et al., 1995; Esler et al., 1996a; Maggio & Mantyh, 1996). To address these issues, an *in vitro* assay for plaque growth using deposition of Aβ at physiological concentrations onto preexisting plaques in unfixed AD cortex sections or homogenates has been developed (Mantyh et al., 1991; Maggio et al., 1992). The use of authentic AD plaques (which contain components other than Aβ itself) further contributes to the physiological relevance of the system. Recent analysis of *in vitro* Aβ deposition reveals that this process is, unlike aggregation, oligomerization-independent (Esler et al., 1996a; Maggio & Mantyh, 1996). Additionally, NMR spectroscopy in water of plaque-competent[3] and plaque-incompetent Aβ congeners (Lee et al., 1995) demonstrates that a particular folded solution conformation correlates with plaque competence. Consequently, it is possible that particular conformational features in soluble Aβ may be necessary to support amyloid deposition in AD. The importance of conformation in other amyloidoses has been reviewed (Kelly, 1996).

Hilbich and co-workers (Hilbich et al., 1992) reported that substitution of two or more hydrophobic amino acid residues between positions 17 and 20 in Aβ results in dramatically increased peptide solubility and altered circular dichroism (CD) spectra. Replacement of single residues in this region of Aβ with proline residues also decreased the aggregation propensity of the peptide (Wood et al., 1995). The work of Tjernberg and co-workers (Tjernberg et al., 1996) and Hughes and colleagues (Hughes et al., 1996) demonstrates that short Aβ peptide fragments with substitutions in the central hydrophobic cluster can alter the assembly of full-length Aβ. Since this region of Aβ is important for both intra- and intermolecular interactions (Inoue et al., 1993), point substitutions in this part of the primary sequence may have important effects on the solution conformation of the peptide and consequently on the rate of plaque growth. In this study, we examine the effect of substitution of threonine for phenylalanine at position 19 in the central hydrophobic cluster of Aβ on both plaque competence and peptide folding in water.

Table 1: Sequences of Aβ Congeners Used in This Study[a]

Aβ Analog	Sequence
Aβ(1-40)-OH	H-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV-OH
Aβ(10-35)-NH_2	H-YEVHHQKLVFFAEDVGSNKGAIIGLM-NH_2
Aβ(10-35)-NH_2 F19T	H-YEVHHQKLVTFAEDVGSNKGAIIGLM-NH_2

[a] The underlined portion indicates the central hydrophobic cluster. Alterations in the primary sequence are indicated in boldface type. H-, free α-amino group; -OH, free α-carboxyl group; -NH_2, α-carboxamide.

EXPERIMENTAL PROCEDURES

Synthetic Peptides. Aβ peptides were purchased (QCB, Inc., Hopkinton, MA) or synthesized by the Merrifield solid-phase methodology (Barany & Merrifield, 1980) using the fluoren-9-ylmethoxycarbonyl (Fmoc)/*tert*-butyloxycarbonyl (Boc) strategy (Meinhofer, 1979). After cleavage from the resin, the crude peptides were purified to homogeneity (>98%) by preparative high-performance liquid chromatography (HPLC). Peptides were stored lyophilized. To ensure that no aggregates were present in the assays, solutions were routinely centrifuged (15000*g* × 10 min) to remove particulates. Peptides were characterized by reverse-phase HPLC (RP-HPLC), laser desorption mass spectrometry, and amino acid analysis (Benson et al., 1981) with satisfactory results in all cases. Except where noted, experiments were performed using analogs of the plaque competent Aβ(10−35)-NH_2 peptide (Lee et al., 1995) to facilitate aqueous NMR experiments. Peptide sequences are shown in Table 1.

Radioiodination. Peptides were radioiodinated as previously described (Maggio et al., 1992). Briefly, Aβ congeners were radiolabeled at tyrosine residue 10 by oxidative iodination using $Na^{125}I$ and Chloramine T. Peptide and unincorporated iodide were separated using reverse-phase adsorption. The oxidized methionine residue at position 35 was reduced from the sulfoxide to the native thioether form using 2-mercaptoethanol. The radioiodinated Aβ congeners were purified to essentially quantitative specific activity (about 2000 Ci/mmol; 10^9 dpm/mg) by RP-HPLC. The radiolabeled peptides were stored at −20 °C in the eluted HPLC mobile phase. 2-Mercaptoethanol (0.5%) was added to all stock tracer solutions to prevent oxidation during storage. Under these conditions, no evidence of oxidation, degradation, or aggregation was detected by RP-HPLC, sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), or size-exclusion chromatography after months of storage. The biochemical properties of Aβ which are important for aggregation, binding, deposition, and template recognition are not significantly affected by the specific incorporation of ^{125}I into the tyrosine residue at position 10

[2] The terms "aggregation" and "assembly" are used in accordance with definitions set forth by the ACS series on aggregation and denaturation (Hermanson, 1979).

[3] "Plaque competent" and "plaque incompetent" are defined as previously described (Lee et al., 1995) and are not used to imply the presence or absence of particular peptides in pathological brain amyloid.

Table 2: Neuropathological Analysis of Cases Used in This Study[a,b]

case:	AD1	AD4	AD5
age/sex	85/F	63/M	92/F
PMI (h)	4	6	22
AD	yes	yes	yes
SP	++	++	+++
CAA	++	++	+++
NFT	+++	++	+++
GVD	+++	+	+++
other	brain atrophy	cortical atrophy	mild cortical atrophy

[a] Neuropathological findings were graded semiquantitatively as absent (−), mild/slight (+), moderate (++), or severe (+++). Diagnosis was in accordance with the criteria of CERAD (Gearing et al., 1995) and of Khachaturian (1986). [b] Abbreviations: CAA, cerebral amyloid angiopathy; F, female; GVD, granulovacuolar degeneration; M, male; NFT, neurofibrillary tangles; PMI, time interval from death to tissue collection; SP, senile plaque.

(Mantyh et al., 1991, 1993; Maggio et al., 1992; Esler et al., 1996a,b; Burdick et al., 1992; Evans et al., 1995).

Tissue. Brain tissue was obtained from normal and AD patients at <15 h post-mortem, snap-frozen in isopentane/liquid nitrogen or on dry ice after collection, and stored below −20 °C until use. The diagnosis of AD was based on the criteria of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Gearing et al., 1995) and those of Khatchaturian (1985), with identification of plaques and subtypes by both classical silver (modified Bielschowsky) stains and anti-Aβ immunohistochemistry (Vinters et al., 1990). The cases used in the present study are summarized in Table 2. Quantitative data reported are from AD case AD1 except where noted. Similar results were obtained for all cases studied (Manyth et al., 1991; Maggio et al., 1992; Esler et al., 1996a). For homogenate binding studies, tissue homogenates were prepared by a modification (Esler et al., 1996a) of the method of Too and Hanley (1988). Briefly, tissue was homogenized (Polytron) in 5 volumes of cold 50 mM Tris-HCl, pH 7.5, containing 10% (w/v) sucrose and protease inhibitors. The homogenates were centrifuged, supernatants were discarded, and the pellets were washed, frozen on dry ice, and stored at −20 °C until use (no longer than 4 months).

Aβ Deposition Assays in Homogenates. Brain membrane homogenate binding was performed as described (Esler et al., 1996a). Briefly, membranes were resuspended and allowed to preincubate for >30 min in 50 mM Tris-HCl (pH 7.5) containing BSA (1 mg/mL), protease inhibitors, and 10 mM $MnCl_2$. ^{125}I-Aβ congener was dissolved in the same buffer (except where noted) and aliquoted to flexible vinyl 96-well assay plates. The membrane suspension was mixed with the radioligand solution at time zero to yield final total Aβ concentrations of 10^{-11}−10^{-9} M and membrane levels of 0.63−10 mg equivalent weight of tissue per well. After the desired incubation time (0.5−4 h), bound tracer and free tracer were separated by centrifugation. Wells containing washed pellets were capped and separated for γ-counting. Control experiments were performed using age-matched plaque-free (non-AD) human cerebral cortex, AD or non-AD cerebellum (unlike cerebral cortex, cerebellum is essentially spared in AD), or no tissue to determine background binding. For active congeners, background in the absence of tissue was less than 5% of the observed deposition onto AD cortex.

Aβ Deposition Assays in Brain Tissue Sections. Deposition assays using AD cortex sections were performed as previously described (Mantyh et al., 1991; Maggio et al., 1992; Esler et al., 1996a; Lee et al., 1995). Briefly, slide-mounted tissue sections were allowed to preincubate for 30 min under the conditions described for homogenate binding experiments. They were then incubated for 2 h with 100 pM labeled Aβ congener. After the desired incubation period, the sections were thoroughly washed, dried, and placed on film with radioiodinated standards. After a 1 week exposure at room temperature (22 °C), the film was developed, fixed, and washed. Results are reported as the product (optical density) × (area covered by the labeled plaques) in the autoradiograms, determined by film densitometry (Maggio et al., 1992). Autoradiography experiments for direct quantitative comparison of Aβ congeners were performed using a single block of tissue with essentially uniform plaque density throughout. Images shown are dark-field autoradiograms generated with tracers of equal specific activity, and with identical film exposure, processing, and image analysis.

Kinetic Analysis of Aβ Deposition. Kinetic plaque growth experiments were performed using the homogenate deposition assay. Time course measurements of Aβ deposition were made at several concentrations for each peptide used. Rates of deposition were determined using linear regression analysis of the time course data and were plotted vs peptide concentration. Linear kinetic plots of rate vs concentration were used for comparison of relative rates while log−log plots were used to determine reaction order.

NMR Sample Preparation. Dry peptide samples were dissolved in 3 mL of 90% H_2O/10% D_2O containing 0.5 mM TSP. The pH (direct meter reading) was adjusted with aqueous HCl or NaOH, and the final volume was adjusted to 3.5 mL. Samples were centrifuged and transferred to 10 mm NMR tubes (Lee et al., 1995). NMR samples were stable at 4 °C for several months in their physical appearance, and the nature and intensity of their ^{1}H spectra. The concentration of each peptide was determined to be about 250 mM by amino acid analysis (Benson et al., 1981).

NMR Spectroscopy. ^{1}H NMR data were collected on Varian UNITY*plus* 750 MHz or Varian UNITY*plus* 500 MHz NMR instruments as previously described (Lee et al., 1995). A 10 mm ^{1}H pulse field gradient (PFG) probe capable of gradients of >70 G/cm was used for all data collection. Briefly, data were collected using a spectral width of 10 or 7 kHz and either 16 384 or 8192 complex points for one-dimensional or two-dimensional spectra, respectively, with a relaxation delay of 1.5 s. The beginning of the relaxation delay was preceded by a purging sequence comprised of a 1 ms gradient of 5 G/cm, a 90° pulse, and a second 1 ms gradient of 13.5 G/cm. Digital resolution in the acquisition dimension was 0.6 and 1.2 Hz/point for one-dimensional and two-dimensional spectra, respectively. All data were acquired at 5°C, and chemical shifts were referenced to internal TSP at 0.00 ppm. Phase sensitive two-dimensional sets were acquired using the TPPI-States method (Marrion et al., 1989). Waveform gradient suppression NOESY (WGS-NOESY) spectra were acquired using a standard 64-step phase cycle with a composite 180° pulse in the middle of the mixing period. A WGS read pulse (Lee et al., 1995) was substituted for the final 90° pulse in the NOESY experiments, and the module was phase-cycled in the standard manner.

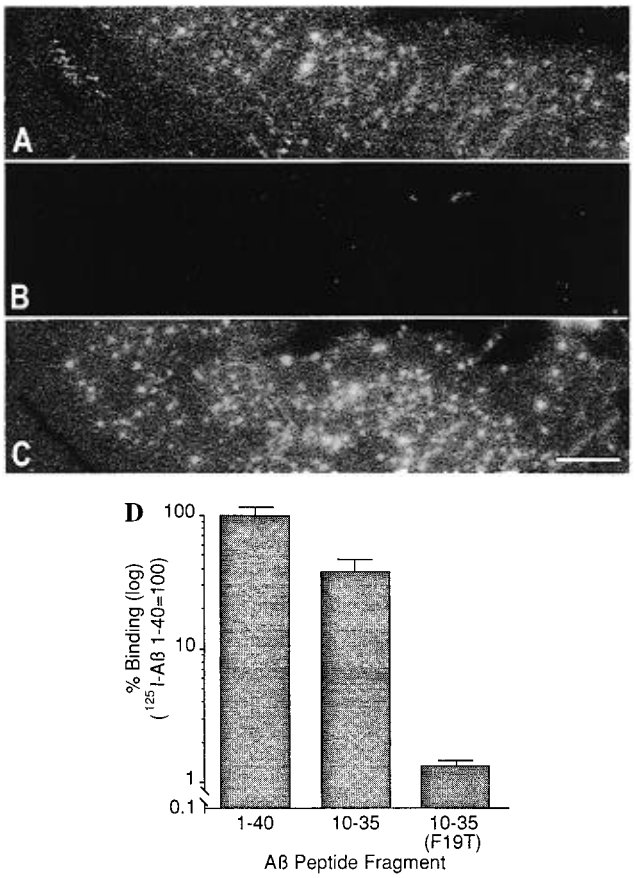

FIGURE 1: Autoradiographic analysis of deposition of Aβ(1−40) and Aβ(10−35)-NH_2 onto human cortical sections. Serial slide mounted sections of human AD cortex were incubated in the presence of 100 pM radiolabeled wild-type Aβ(10−35)-NH_2 (A), Aβ(10−35)-NH_2 F19T (B), or Aβ(1−40) (C), as described in the text. Similar experiments were performed using age-matched plaque-free (non-AD) control tissue with 100 pM wild-type Aβ(10−35)-NH_2 or Aβ(1−40) (not shown). The dark-field autoradiographs shown were obtained with parallel exposure and development to allow for quantitative comparison by densitometry (D). Light areas represent sites of radiolabeled Aβ deposition (A−C) and correspond to amyloid sites visible by thioflavin S and Congo red staining or anti-Aβ immunohistochemistry (Maggio et al., 1992). Results using tissue from case AD5 are shown. Scale bar = 0.75 mm.

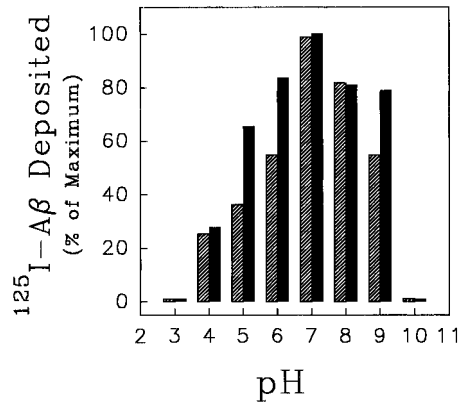

FIGURE 2: pH dependence of radiolabeled Aβ(1−40) and Aβ(10-35)-NH_2 deposition onto AD brain tissue amyloid. Solutions of 100 pM radiolabeled Aβ(10−35)-NH_2 (shaded bars) or wild-type Aβ(1−40) (solid bars) were incubated with AD cortex sections (case AD4) or homogenates (case AD1) (Esler et al., 1996a) for 2 h in 50 mM buffers over a wide pH range. Several overlapping buffer systems were used as previously described (Esler et al., 1996a) to provide adequate buffering over the entire pH range. Results are reported as the percent of maximum deposition observed for each peptide. Results are pooled from four separate experiments that gave similar results. Points represent at least three determinations with SEM ≤5% of the mean. As deposition is a nonsaturable process (Maggio & Mantyh, 1996), ordinate values represent rates, not equilibrium.

RESULTS

Aβ(10−35)-NH_2 and Aβ(1−40) Deposit Similarly onto Amyloid in AD Cortex. In order to generate a model system for Aβ structure−activity studies, a truncated plaque-competent Aβ congener with greater solubility than the full-length Aβ was developed (Lee et al., 1995). To ensure that the selected peptide congener retained plaque growth competence, radioiodinated peptides were used to measure Aβ congener deposition onto authentic AD plaques. Consistent with previous work (Lee et al., 1995), the Aβ congener Aβ(10−35)-NH_2 deposited onto tissue amyloid in AD cortical sections (Figure 1A) in a specific and nonsaturable manner and is hence "plaque competent". In all cases, visualization of binding sites for ^{125}I-Aβ(10−35)-NH_2 by autoradiography in conjunction with amyloid subtype identification showed that the peptide deposited on parenchymal amyloid plaques and vascular sites in the AD brain (not shown). Thus, in the AD cerebral cortex, the Aβ(10−35)-NH_2 congener (Figure 1A), like Aβ(1−40) (Figure 1C), was deposited ontocerebrovascular amyloid and compact, diffuse, and neuritic plaques; in the latter case, both the core and halo of essentially every plaque were labeled (not shown). The labeled Aβ(10−35)-NH_2 congener retained approximately 40% of the deposition activity of Aβ(1−40) (Figure 1D). In contrast to the AD cortex, in which a significant fraction of the affected gray matter area was occupied by plaques competent of binding Aβ(1−40) or Aβ(10−35)-NH_2, essentially no deposition was observed in non-AD cortex, AD cerebellum, or non-AD cerebellum (Mantyh et al., 1991; Maggio et al., 1992; Esler et al., 1996a) at all peptide concentrations and times tested. No significant oxidation or degradation of peptide solutions exposed to tissue was observed by RP-HPLC.

In order to investigate the biochemical similarity between Aβ(10−35)-NH_2 and the full-length wild-type peptide, the pH and kinetic profiles of deposition were examined. Deposition of Aβ(1−40) (solid bars) or Aβ(10−35)-NH_2 (shaded bars) onto plaques in AD cortical sections and homogenates was found to be a pH-dependent process with a maximum around pH 7.0 (Figure 2). For both peptides, there was essentially no deposition below pH 4 or above pH 9. At a pH of 5.6 (the pH used in NMR studies), a significant fraction (40−90%) of the maximum deposition activity was retained for both the truncated and full-length peptides (Figure 2).

As was previously shown for the full-length wild-type Aβ(1−40) (Esler et al., 1996a), deposition of the Aβ(10−35)-NH_2 congener onto plaques in AD cortex homogenates increased linearly with time with no evidence of a "lag time" (Jarrett & Lansbury, 1993) over 4 h (Figure 3A). Significantly greater rates of deposition were observed with increases in Aβ(10−35)-NH_2 concentration. When the rate of deposition (as determined from slopes of time course experiments) was plotted vs Aβ(10−35)-NH_2 concentration, first-order dependence was evident (Figure 4, open symbols). A log−log plot of deposition rate vs peptide concentration

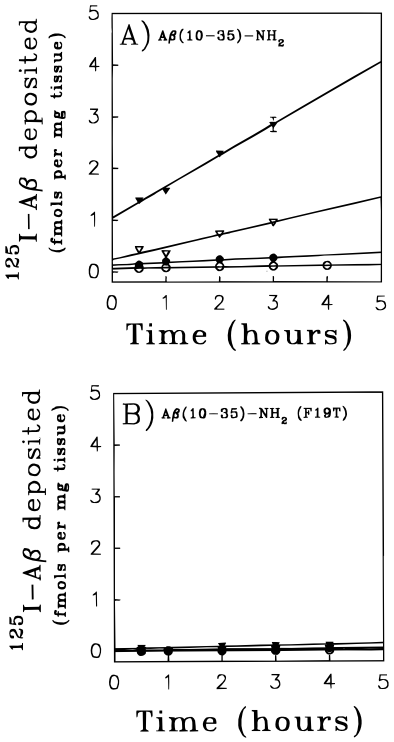

FIGURE 3: Time course of Aβ(10-35)-NH_2 congener deposition onto AD cortex. Time course experiments were performed for both the wild-type analog and the F19T analog over a wide range of concentrations. Peptide solutions at or around physiological concentration were incubated with AD cortex homogenates for times ranging form 0.5 to 4.0 h. (A) ^{125}I-Aβ(10−35)-NH_2 solutions of 33 pM (○), 100 pM (●), 300 pM (▽), and 900 pM (▼) were used. (B) ^{125}I-Aβ(10−35)-NH_2 F19T solutions of 30 pM (○), 80 pM (●), 220 pM (▽), and 600 pM (▼) were used. The amount of labeled peptide deposited was determined as described in the text. Error bars represent the SEM of six determinations. Error bars that are not visible are smaller than the area covered by the symbol.

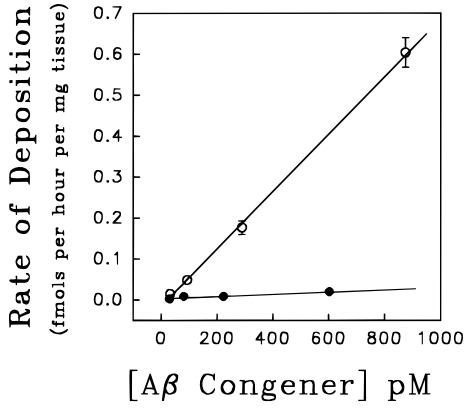

FIGURE 4: Initial rate of Aβ(10−35)-NH_2 congener deposition onto AD cortex homogenates vs peptide concentration. The initial rate of deposition was determined for ^{125}I-Aβ(10−35)-NH_2 (empty symbols) or ^{125}I-Aβ(10−-35)-NH_2 F19T (solid symbols) via linear regression analysis of time course data presented in Figure 2. The initial rates of deposition were determined for four concentrations of each analog. Error bars shown represent the error on the rates of deposition as determined via regression analysis.

displays linear dependence ($r^2 = 0.99$) with a slope of 1.1 (not shown).

Aβ(10−35)-NH_2 F19T Is Inactive. We have previously shown that plaque competence correlates with folding in two Aβ congeners (Lee et al., 1995). In order to further test the hypothesis that folding around the hydrophobic cluster of the peptide is important for plaque competence, we synthesized an Aβ analog predicted to alter folding. Since the central hydrophobic cluster of Aβ (residues 17−21) displays significantly folded conformation at pH values where the peptide is plaque-competent (Lee et al., 1995) and mutation of multiple residues in this region altered CD spectra (Hilbich et al., 1992), we synthesized an F19T-substituted Aβ(10−35)-NH_2 congener (Table 1), placing a single point substitution in the center of the hydrophobic cluster. In contrast to the wild-type Aβ(10−35)-NH_2 (Figures 3A and 1A), the substituted peptide Aβ(10−35)-NH_2 F19T (Figures 3B and 1B) did not show significant deposition onto AD cortex. The low level of deposition of the F19T analog onto AD cortex was not significantly greater than the deposition of the wild-type Aβ(10−35)-NH_2 onto control tissue (not shown) in this assay. When time course experiments were performed, levels of F19T-substituted congener deposition did not substantially increase with time (Figure 3B) or concentration (Figures 3B and 4, solid symbols) in contrast to the wild-type peptide (Figures 3A and 4, open symbols).

F19T Analog Is Less Folded than Wild-Type Aβ(10−35)-NH_2. In order to determine if the loss of plaque competence via the F19T-substitution correlates with a change in peptide folding, NMR experiments were performed with the active Aβ(10−35)-NH_2 and the inactive F19T-substituted analog in water at pH 5.6. Nuclear Overhauser enhanced spectroscopy (NOESY) NMR experiments were performed on both analogs. Figure 5 shows a comparison of an expanded region of the NOESY spectra from the wild-type Aβ(10−35)-NH_2 peptide (Figure 5B) and the same region for the F19T-substituted analog (Figure 5A) under similar experimental conditions. It is evident from the number and intensity of NOE cross-peaks present in each of the expansions that Aβ(10−35)-NH_2 exists in a significantly more folded conformation compared to Aβ(10−35)-NH_2 F19T. Within this region of the spectra, the wild-type congener (Figure 5B) contains more than 30 detectable NOE cross-peaks while the F19T-substituted peptide (Figure 5A) has only 6 such cross-peaks detectable in the same region. Figure 6 shows slices through the two-dimensional NOESY spectra corresponding to the ortho aromatic protons of residue Phe 20. It is clear from the number and intensity of the NOE peaks present that the wild-type sequence (Figure 6B) has a more folded conformation than the F19T analog (Figure 6A). In the region of 9.0−8.0 ppm, at least seven strong NOE cross-peaks are present in the wild-type peptide (Figure 6B) while only two are visible in the F19T-substituted peptide (Figure 6A). Similarly, in the region of 4.5−1.0 ppm, the plaque-competent Aβ(10−35)-NH_2 peptide contains at least 10 intense cross-peaks while the comparable region of F19T Aβ(10−35)-NH_2 contains just three clear intraresidue cross-peaks across a well-defined base line as would be expected for a mobile side chain (Figure 6). A more detailed comparision of conformational differences between Aβ(10−35)-NH_2 and Aβ(10−35)-NH_2 F19T will be reported elsewhere.

DISCUSSION

The Aβ(10−35)-NH_2 model peptide previously described (Lee et al., 1995) appears to be a good model system for probing structure−function relationships in Aβ plaque growth. The morphological pattern obtained for Aβ(10−35)-NH_2 deposition onto amyloid plaques in AD brain sections is similar to that observed with the full-length

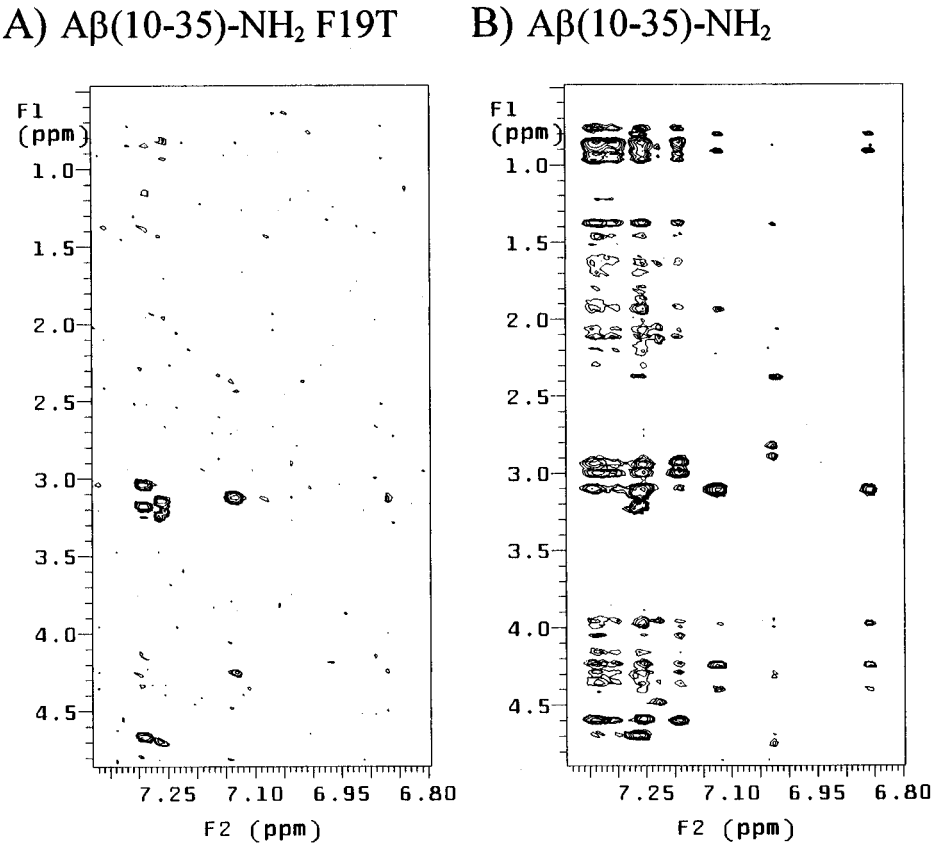

FIGURE 5: Expansion of the NOESY spectrum of wild-type Aβ(10−35)-NH_2 and Aβ(10−35)-NH_2 F19T. The same region of the NOESY spectra for wild-type Aβ(10−35)-NH_2 (B) and Aβ(10−35)-NH_2 F19T (A) is illustrated. The greater number and intensity of NOE cross-peaks present in the wild-type (B) compared with the mutant (A) sequence reflect the greater degree of folding in the wild-type peptide.

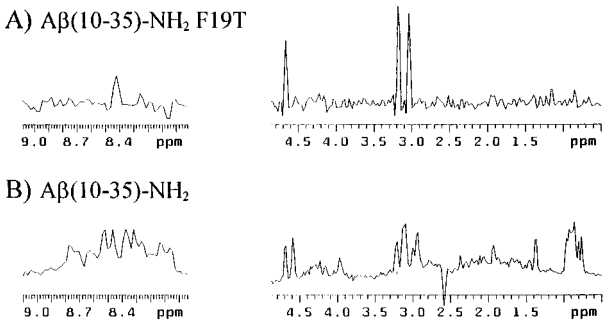

FIGURE 6: Slices through the NOESY spectra of wild-type Aβ(10−35)-NH_2 and Aβ(10−35)-NH_2 F19T at the F2 frequency corresponding to the aromatic ortho protons (H2, H6) of Phe 20 (Lee et al., 1995). Slices were taken through proton NOESY spectra of the wild-type Aβ congener (B) and F19T-substituted Aβ analog (A) shown in Figure 5.

peptide (Figure 1). In addition, deposition of Aβ(10−35)-NH_2 (Figures 2−4) and Aβ(1−40) (Figure 2) (Esler et al., 1996a) share common pH−activity profiles and kinetic order, suggesting that the process of plaque growth by deposition of these peptides is biochemically similar. Hence, neither the N-terminal (residues 1−9) nor the C-terminal (residues 36−42) region of Aβ is necessary for plaque competence. These structure−activity results for Aβ deposition are in sharp contrast with those reported for Aβ aggregation. The C-terminal region of Aβ (residues 39−42) appears to be important in the aggregation propensity of the peptide (Jarrett & Lansbury, 1992, 1993; Jarrett et al., 1993, 1994). Because Aβ(10−35)-NH_2 is more soluble (unpublished results) than the full-length peptide, and thus more amenable to NMR experiments in water, and retains a significant fraction of plaque deposition activity, the 26-mer peptide is a good model for studying the effect of sequence substitutions on Aβ conformation and activity under similar experimental conditions. Combined, these methods show promise in elucidating some of the key molecular and structural requirements for Aβ deposition and thus may be useful in identifying targets for slowing the process of amyloidosis in AD.

The distinction between Aβ aggregation and deposition is further illustrated by differences in time dependence, pH optimum, and kinetic order (Esler et al., 1996a; Maggio & Mantyh, 1996). Since aggregation is a nucleation-dependent process, it is characterized by both a lag time and high-order kinetic dependence on Aβ concentration (Jarrett & Lansbury, 1992, 1993; Jarrett et al., 1993, 1994; Lomakin et al., 1996). In contrast, deposition of physiological concentrations of Aβ onto a preexisting template has a linear time course (Mantyh et al., 1991; Maggio et al., 1992; Esler et al., 1996a) and shows first-order dependence on Aβ concentration (Esler et al., 1996a). While Aβ aggregation shows a pH optimum around the isoelectric point of the peptide at ≈5 (Burdick et al., 1992), Aβ deposition onto AD cortex or synthetic Aβ fibrils has a pH optimum of ≈7 (Figure 2) (Esler et al., 1996a; Maggio & Mantyh, 1996; Naiki & Nakakuki, 1996). Thus, the differences in kinetic order, pH optimum, time dependence, and structure−activity relations lead us to conclude that plaque growth by Aβ deposition and amyloid formation by Aβ aggregation are mechanistically distinct. This distinction may have significant implications for the progression and treatment of AD.

Although the relative importance of amyloid template formation and growth to neuropathology is difficult to assess,

quantitative pathological observations suggest that *in vivo*, as well as *in vitro*, the processes are distinct (Hyman et al., 1995). Quantitative analysis of AD brain reveals a log-normal distribution of plaque size (Hyman et al., 1995), implying a porous template for Aβ deposition. Thus, increasing the mass of an existing amyloid plaque by deposition of Aβ would not necessarily increase its diameter; instead, the density may increase during the maturation process. The density of mature senile plaques rather than the total number of immunopositive Aβ foci correlates with the clinical state (Selkoe, 1994), strongly suggesting that amyloid maturation is a key process in AD.

Since plaque competence appears to correlate with folding (Lee et al., 1995) and recent work suggests that the central hydrophobic cluster of Aβ may be important in directing this folding (Casey et al., 1996), we synthesized an Aβ congener designed to alter folding in this region. The work of Hilbich and colleagues (Hilbich et al., 1992) and Wood and colleagues (Wood et al., 1995) suggests that substitution of two or more hydrophobic amino acids in this region can cause an alteration in the peptide solution conformation and greatly increase solubility. The central amino acid in the hydrophobic cluster, F19, was replaced by threonine in an attempt to perturb the peptide conformation by altering the steric nature and polarity of the side chain. Our previous work suggests that a single amino acid substitution of F by T could cause an alteration in folding of an Aβ(12−28) analog (Casey et al., 1996). Since a point substitution was sufficient to alter the structural characteristics of the Aβ(12−28) analog (Casey et al., 1996), we reasoned that a comparable substitution in the plaque-competent Aβ(10−35)-NH_2 might also alter the solution conformation. Structural studies in conjunction with plaque growth experiments on the wild-type peptide and the F19T-substituted analog may enhance our understanding of the importance of this region for Aβ conformation and deposition rate. The importance of conformation and conformational transitions in other amyloidogenic peptides and proteins has been reviewed (Kelly, 1996).

Unlike the wild-type 26-mer, which is capable of depositing onto Aβ plaques in AD cortex in a manner similar to the full-length Aβ, the F19T-substituted analog is inactive. Whereas Aβ(10−35)-NH_2 shows a linear increase in deposition with time and first-order kinetic dependence on peptide concentration, the F19T-substituted analog shows no substantial increase in deposition with time or concentration.

NMR NOESY experiments in water illustrate that the wild-type Aβ(10−35)-NH_2 has a more folded conformation (on the NMR time scale) at pH 5.6 than Aβ(10−35)-NH_2 F19T. Consistent with our previous findings that Aβ(10−35)-NH_2 is folded under conditions where the molecule is plaque-competent and less folded under conditions where the peptide is plaque-incompetent (Lee et al., 1995), the less folded F19T peptide is inactive. These results are significant for several reasons. As was observed for the Aβ(12−28) model peptide (Casey et al., 1996), the central hydrophobic cluster appears also to be important for directing folding of the longer Aβ(10−35)-NH_2 molecule. Thus, single alterations in the Aβ primary sequence can affect Aβ activity and folding. Because these results are consistent with previous work relating Aβ folding to Aβ plaque competence (Lee et al., 1995), the importance of this region for deposition is underscored. Furthermore, these data suggest that the association of solution Aβ with an amyloid template may involve docking interactions of specific regions of the peptide or particular amino acids.

While other groups have identified the importance of the central hydrophobic cluster (Hilbich et al., 1992; Wood et al., 1995) for Aβ solubility, an important role for this region in β-amyloid deposition is identified here. Models designed to fit Aβ fiber diffraction patterns also indicate that the central hydrophobic cluster is important in stabilizing intermolecular contacts in fibrils (Fraser et al., 1991a,b; Inoue et al., 1993).

Since the central hydrophobic cluster appears to be important for stabilizing intra- and intermolecular interactions involved in aggregation, deposition, and amyloid formation, a pharmacophore designed to alter folding in this region of Aβ may show promise in reducing plaque formation and slowing plaque growth and progression of the disease. As more high-resolution structural information about Aβ becomes available, this region may become an excellent target for structure-based drug design.

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