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Molecular Determinants of Amyloid Deposition in Alzheimer's Disease: Conformational Studies of Synthetic β -Protein Fragments[†]

Kurt Halverson,[‡] Paul E. Fraser,^{§,||} Daniel A. Kirschner,[§] and Peter T. Lansbury, Jr.*[†]

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, Neurology Research, Children's Hospital, Boston, Massachusetts 02115, and Department of Neurology, Harvard Medical School, Boston, Massachusetts 02115

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ABSTRACT: The amyloid β -protein (1-42) is a major constituent of the abnormal extracellular amyloid plaque that characterizes the brains of victims of Alzheimer's disease. Two peptides, with sequences derived from the previously unexplored C-terminal region of the β -protein, β 26-33 ($\text{H}_2\text{N-SNKGAIIG-CO}_2\text{H}$) and β 34-42 ($\text{H}_2\text{N-LMVGGVVIA-CO}_2\text{H}$), were synthesized and purified, and their solubility and conformational properties were analyzed. Peptide β 26-33 was found to be freely soluble in water; however, peptide β 34-42 was virtually insoluble in aqueous media, including 6 M guanidinium thiocyanate. The peptides formed assemblies having distinct fibrillar morphologies and different dimensions as observed by electron microscopy of negatively stained samples. X-ray diffraction revealed that the peptide conformation in the fibrils was cross- β . A correlation between solubility and β -structure formation was inferred from FTIR studies: β 26-33, when dissolved in water, existed as a random coil, whereas the water-insoluble peptide β 34-42 possessed antiparallel β -sheet structure in the solid state. Solubilization of β 34-42 in organic media resulted in the disappearance of β -structure. These data suggest that the sequence 34-42, by virtue of its ability to form unusually stable β -structure, is a major contributor to the insolubility of the β -protein and may nucleate the formation of the fibrils that constitute amyloid plaque.

The brains of individuals with Alzheimer's disease (AD)¹ and Down's syndrome are characterized by the presence of abnormal extracellular amyloid plaques and cerebrovascular amyloid (Glenner, 1988). The plaque core is primarily composed of a single 42 or 43 amino acid protein (see Figure 1), known as the amyloid β -protein (Glenner & Wong, 1984; Masters et al., 1985; Kang et al., 1987), which is extremely difficult to solubilize. Cerebrovascular amyloid has been shown to contain a more soluble form of the β -protein which corresponds to residues 1-39 (Prelli et al., 1988) or 1-40 (Joachim et al., 1988). The β -proteins are derived from a precursor protein of ca. 91 kDa, the amyloid precursor protein (APP), which is produced in several forms by alternative mRNA splicing (Kang et al., 1987; Dyrks et al., 1988; Ponte et al., 1988; Tanaka et al., 1988). Attempts to correlate expression levels of the various forms of APP with the deposition of amyloid have been inconclusive (Ponte et al., 1988; Tanaka et al., 1988). The APP may be a cell-surface protein; the amyloid β -protein sequence overlaps the putative transmembrane sequence of the precursor [the proposed outer membrane border (Kang et al., 1987) of APP is between Lys²⁸ and Gly²⁹ (see Figure 1)]. The β -protein, which may be generated in AD patients due to a defective proteolytic pathway and/or membrane damage (Dyrks et al., 1988; Carrell, 1988; Weidemann et al., 1989), may be an intrinsically insoluble protein. A synthetic peptide representing the N-terminal 28 amino acids of the β -protein (1-28) has neurotrophic activity in vitro (Whitson et al., 1989); however, expression of an APP frag-

ment containing the β -protein sequence is toxic to neurons in culture (Yankner et al., 1989). These findings suggest that the β -protein may be a normal soluble cellular protein. Precipitation of the β -protein may occur in the abnormal microenvironment that characterizes brain pathology [e.g., $[\text{Al}^{3+}]$ (Candy et al., 1986), pH, temperature]. We will refer to the environmental effects on folding as extrinsic, as opposed to intrinsic effects that derive directly from the chemical structure of the protein. Extrinsic effects on protein precipitation may be important in systemic amyloidosis (Castaño & Frangione, 1988) and cataract formation (Masters et al., 1977) and may lead to inclusion body formation by recombinant proteins (Kane & Hartley, 1988). The goal of our research is to elucidate, at the molecular level, the intrinsic and extrinsic factors that lead to aggregation and precipitation of the β -protein.

Fibrillar amyloid, isolated from AD brain, has been shown by X-ray diffraction to contain protein in the cross- β conformation (Kirschner et al., 1986). In this conformation of stacked pleated β -sheets, the fiber axis is perpendicular to the polypeptide β -strand and parallel to the hydrogen-bond direction (Marsh et al., 1955; Geddes et al., 1968). The portion(s) of the β -protein which are involved in β -sheet formation has (have) yet to be determined. A synthetic peptide corresponding to the amino-terminal end of the amyloid β -protein (β 1-28) forms cross- β -fibrils, which morphologically resemble those of the amyloid β -protein (Kirschner et al., 1987). It has been suggested that the sequence 11-24 of the amyloid β -protein (see Figure 1) contains the critical intrinsic information that specifies cross- β -fibril formation (Kirschner et al., 1987). In light of the fact that cerebrovascular amyloid (1-39/40)

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[‡] Massachusetts Institute of Technology.

[§] Children's Hospital and Harvard Medical School.

^{||} Medical Research Council of Canada Postdoctoral Fellow.

¹ Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EM, electron microscopy; FAB, fast atom bombardment; HFIP, hexafluoro-2-propanol; THF, tetrahydrofuran; TFMSA, trifluoromethanesulfonic acid; TFA, trifluoroacetic acid.

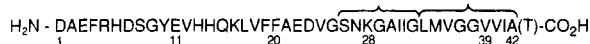


FIGURE 1: β -Protein from amyloid plaque core. Cerebrovascular amyloid β -protein consists of amino acids 1–39 or 1–40. The sequences corresponding to synthetic peptides β 26–33 and β 34–42 are indicated.

is reportedly more soluble than amyloid plaque core (1–42/43) (Prelli et al., 1988; Joachim et al., 1988), we chose to investigate the role of the hydrophobic C-terminal region of the β -protein in the formation and stabilization of the cross- β -fibril.

To obtain adequate amounts of the β -proteins for structural studies, we have undertaken a total synthesis of the 42 amino acid plaque β -protein and the 39 amino acid cerebrovascular β -protein using a convergent solid-phase fragment-coupling methodology (Kaiser, 1989; Kaiser et al., 1989). We report herein our investigations of two synthetic peptides that were prepared as intermediates in the synthesis of the plaque β -protein. These peptides comprised amino acids 26–33 and 34–42, respectively, of the plaque amyloid β -protein. Conformational studies (FTIR, X-ray diffraction) of these peptides as well as their solubility properties suggest that the C-terminal sequence is responsible for the insolubility of the β -protein and may be important in directing the deposition of amyloid plaque.

MATERIALS AND METHODS

Synthesis and Purification of Peptides. Peptides β 26–33 and β 34–42 (see Figure 1) were synthesized on the Kaiser oxime resin according to standard procedures (DeGrado & Kaiser, 1980, 1982). The resin was prepared as reported (Findeis & Kaiser, 1989). All reagents used in the peptide synthesis were purchased from commercial sources with the exception of *N*-hydroxypiperidine, which was prepared by a published procedure (Sabel, 1966). Serine was protected as the benzyl ether; lysine, as the chlorobenzyl carbamate. Diisopropylethylamine was distilled from ninhydrin at reduced pressure. Protected peptides were purified by reversed-phase (RP) HPLC on a Waters Delta-Pak C4 column (19 mm \times 300 mm, 100-Å pore size, 15- μ m particle size). Deprotection of peptide β 26–33 was accomplished by treatment with trifluoromethanesulfonic acid (15% by volume)–trifluoroacetic acid (75%) (Tam et al., 1986) containing ethanediol (3%) and thioanisole (7%) as scavengers [0 \rightarrow 23 $^{\circ}$ C, 25 min, 1.35 mL/100 mg of peptide (Applied Biosystems User Bulletin, June 1987)]. Acid was removed by evaporation, and the residue was dissolved in acetic acid–water. Extraction with chloroform followed by gel filtration (Pharmacia LH-20, DMF) was adequate to remove most of the residual scavenger. Peptide β 34–42 was treated with TFMSA–TFA containing dimethyl sulfide to reduce methionine sulfoxide (Tam et al., 1986). Solvent was removed, and the residue was triturated with ether. The crude free peptides were then purified by RPHPLC. The purity of each peptide was verified by analytical RPHPLC (C4, 3.9 mm \times 30 cm) under isocratic elution conditions.

Characterization of Peptides. The purified protected and deprotected peptides were analyzed by hydrolysis (1:1 propionic acid–HCl, 3 h at 130 $^{\circ}$ C, or 6 N HCl, 24 h at 110 $^{\circ}$ C) followed by amino acid analysis (Waters Pico-Tag). Reported numbers for Ser and Met are uncorrected for decomposition. Each compound was also analyzed by FABMS (nitrobenzyl alcohol matrix) to determine molecular weight and by tandem FAB–collision MS to determine sequence (Biemann, 1986). Proton NMR spectra were obtained for each peptide (Varian XL-300). Fourier-transform infrared spectra were measured on a Mattson Cygnus 100-V spectrometer using 0.015-mm CaF₂ cells. Solvent subtraction methods were utilized for samples in HFIP, and spectra in D₂O were base line corrected.

Peptide solubility was tested by addition of 2 mg of peptide to 150 μ L of each solvent. After being heated for 15 min at 60 $^{\circ}$ C with intermittent sonication, the sample was centrifuged, and a measured aliquot of supernatant was removed and filtered. The concentration of peptide in the supernatant was determined by quantitative amino acid analysis. Lithium bromide was dried by heating under vacuum, and a 2 M solution in tetrahydrofuran (distilled from sodium and benzophenone) was made and stored under a nitrogen atmosphere.

Electron Microscopy. Aliquots of 1–2 mg/mL aqueous peptide suspensions were placed on pioloform-coated, glow-discharged copper grids and stained with 2% (w/v) uranyl acetate. Samples were viewed in a JOEL 100-S electron microscope operating at 80 kV. Micrograph calibrations were made with tropomyosin paracrystals (kindly provided by Dr. C. Cohen, Brandeis University, Waltham, MA).

X-ray Diffraction. Lyophilized and solubilized samples were studied. Peptides were dissolved in 10% formic acid and slowly air-dried or solubilized in HFIP which was evaporated under wet nitrogen to eliminate the possibility of sulfoxide formation. The starting peptide concentration was ca. 15 mg/mL. Evaporation was carried out in 0.8 mm diameter glass capillaries which were siliconized.

X-ray experiments utilized Ni-filtered and double-mirror-focused Cu K α radiation from an Elliott GX-20 rotating-anode generator (GEC Avionics, Hertfordshire, England) operated at 35 kV and 35 mA. Patterns were recorded on Kodak DEF X-ray film with exposure times of 1–3 days. Specimen to film distances were 69.6 and 87.7 mm as calibrated from known standards. Diffraction spacings were measured directly from the X-ray film.

RESULTS

Synthesis, Purification, and Characterization of Peptides β 26–33 and β 34–42. The two peptides were synthesized on the Kaiser oxime resin according to published procedures (Kaiser, 1989; Kaiser et al., 1989). In both cases, the substitution level of resin-bound protected peptides was 0.2–0.25 mmol/g of resin. Both peptides were cleaved from resin with *N*-hydroxypiperidine (Nakagawa & Kaiser, 1983). After zinc/acetic acid cleavage of the resultant hydroxypiperidyl ester (Nakagawa & Kaiser, 1983), the protected 8-mer [Boc-Ser(Bzl)-Asn-Lys(2-ClBzl)-Gly-Ala-Ile-Ile-Gly-CO₂H] was precipitated, washed thoroughly with water, and purified by RPHPLC (isocratic elution; 51% H₂O–49% CH₃CN, 0.1% acetic acid). A 43% purified yield of protected peptide (based on resin-bound glycine) was obtained. The protected peptide had an amino acid analysis consistent with the proposed structure.² The purity of the material was verified by analytical RPHPLC (55% H₂O–45% CH₃CN, 0.1% acetic acid; *V_R* = 16 mL). A pair of molecular ion peaks consistent with the calculated mass were observed by FABMS.² In addition, a FABMS–collision tandem mass spectrum verified the amino acid sequence (Biemann, 1986). Finally, the proton NMR spectrum obtained was consistent with the expected composition.² Deprotection of the 8-mer was accomplished with a TFMSA–TFA mixture containing ethanedithiol and thio-

² Protected β 26–33. Amino acid analysis: S, 0.5 (1 expected); N, 1.1 (1); K, 0.8 (1); G, 2.0 (2); A, 1.0 (1); I, 2.2 (2). FABMS: 1117.8 (M + H⁺), 1139.8 (M + Na⁺); MW = 1116.5. ¹H NMR (DMSO, 300 MHz): δ 8.2 (m, 3 H), 8.08 (d, 1 H, *J* = 6.2 Hz), 7.89 (d, 1 H, *J* = 9 Hz), 7.79 (d, 1 H, *J* = 7 Hz), 7.69 (d, 1 H, *J* = 9 Hz), 7.48 (m, 3 H), 7.3 (m, 8 H), 7.06 (s, 1 H, Asn), 6.98 (d, 1 H, *J* = 8 Hz), 5.1 [s, 2 H, Lys(ClZ)], 4.55 (m, 1 H), 4.5 [s, 2 H, Ser(Bzl)], 4.2 (m), 2.95 (m, 2 H), 2.5 (m, 2 H), 1.7 (br m, 4 H), 1.4 (s, 9 H, BOC), 1.2 (d, 3 H, *J* = 6.6 Hz, AlaCH₃), 1.1 (m), 0.8 (m, 12 H, IleCH₃).

Table I: Solubility Characteristics of Peptides β 26–33 and β 34–42^a

solvent	β 26–33 (H ₂ N-SNKGAIIG-CO ₂ H)	β 34–42 (H ₂ N-LMVGGVVIA-CO ₂ H)	amyloid plaque core ^b
methylene chloride	I	I	nd
dimethylformamide (DMF)	S	SS	nd
trifluoroethanol (TFE)	S (9 mg/mL)	I	nd
phenol	SS	I	+
hexafluoro-2-propanol (HFIP)	S	S (25 mg/mL)	nd
2M LiBr-THF	S	S (≥ 100 mg/mL)	nd
acetic acid	S	I	–
formic acid	S	S (≥ 50 mg/mL)	+
water (pH 6.3)	S	I	–
1 M NaCl (pH 6.3)	S	I	nd
1 M NaCl (pH 3.0)	S	I	nd
PBS (pH 7.2)	S	I	nd
5% octyl glucoside	nd	I	nd
6 M guanidinium thiocyanate	S	SS	+
6 M guanidinium chloride	S	I	–
1 M NaOH	S	I	+
$\langle P_\alpha \rangle - \langle P_\beta \rangle$ ^{c,d}	–0.10	–0.21	–0.03 (β 1–42)
$\langle P_c \rangle$ ^d	1.08	0.83	0.96 (β 1–42)

^aS, soluble at 23 °C with sonication (≥ 30 mg/mL, unless indicated); SS, slightly soluble, requires heating (60 °C, 1–2 mg/mL); I, insoluble at 60 °C with sonication (≤ 0.2 mg/mL); nd, not determined. ^bThis material contains β 1–42 and several truncated analogues. Qualitative (+/–) solubility information was reported (Masters et al., 1985). ^cA peptide derived from amino acids 11–24 (β 11–24) would have the following values: $\langle P_\alpha \rangle - \langle P_\beta \rangle = 0.10$; $\langle P_c \rangle = 0.82$. Peptide β 1–28: $\langle P_\alpha \rangle - \langle P_\beta \rangle = 0.07$; $\langle P_c \rangle = 0.99$. ^dChou & Fasman, 1978.

anisole. Precipitation of the deprotected product with ether, as recommended in the literature (Tam et al., 1986), afforded β 26–33 as a white solid, which we were unable to resolubilize. We therefore routinely remove scavenger by extraction followed by gel filtration (LH-20). This peptide was purified by RPHPLC [85% H₂O–15% (5% TFE in CH₃CN), 0.1% TFA; isocratic elution]. The purified peptide [H₂N-SNKGAIIG-CO₂H, β 26–33] was obtained in 54% yield. β 26–33 had an amino acid analysis, ¹H NMR, and FABMS (M⁺ and collision sequence) consistent with the proposed structure.³

The protected 9-mer (Boc-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-CO₂H) was extremely difficult to solubilize; therefore, the crude material was deprotected (trifluoroacetic acid, 0 °C, 30 min) to afford peptide β 34–42. Peptide β 34–42 was purified by RPHPLC [gradient elution: 5 min at 90% H₂O–10% (95:5 CH₃CN–trifluoroethanol), 0.1% acetic acid; linear gradient to 80:20 (5 min); linear gradient to 30:70 (15 min)]. We found that the presence of trifluoroethanol in the organic phase was necessary to obtain acceptable recoveries of peptide off the C4 column. In addition, the recovery was extremely sensitive to the amount of peptide loaded on the column. A 40% yield of pure β 34–42 (based on resin-bound alanine) was obtained. Purity was verified by RPHPLC [isocratic elution; 85% H₂O–15% (95:5 CH₃CN–trifluoroethanol), 0.1% TFA; $V_R = 21$ mL]. The experimentally determined amino acid composition (amino acid analysis, FABMS M⁺, ¹H NMR) was consistent with the proposed structure.⁴ Peptide β 34–42 was quite susceptible to oxidation of methionine to provide the methionine sulfoxide containing compound. The identity of the oxidized material was verified

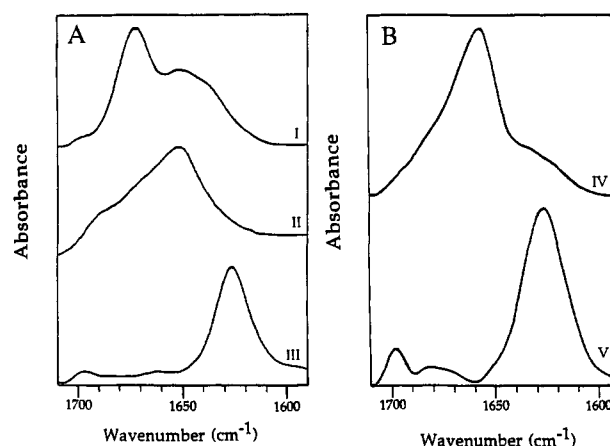


FIGURE 2: FTIR absorption spectra: (A) solution of β 26–33 in D₂O (I, trifluoroacetate salt at ca. 1670 cm^{–1}), solution of β 34–42 in HFIP (II, trifluoroacetate salt), and precipitated β 34–42 (III, acetate salt); (B) solution of β 34–42 in 2 M LiBr-THF (IV) and same sample after precipitation by addition of trace D₂O (V).

by FABMS–collision MS (Biemann, 1986). The methionine sulfoxide containing peptide could be separated from peptide β 34–42 by RPHPLC (above conditions; $V_R = 19$ mL); however, the mixture was routinely reduced (Tam et al., 1986) prior to preparative HPLC.

Peptide β 34–42 Has Limited Solubility. Data concerning the solubility of β 26–33 and β 34–42 (this study) and the amyloid plaque cores (Masters et al., 1985) are summarized in Table I. Information for organic solvents commonly used in peptide synthesis is included. Both peptides showed fair solubility in hexafluoro-2-propanol (HFIP), which is known to be an excellent hydrogen-bond donor (Narita et al., 1988). Clearly, β 26–33 is much more soluble in organic and in aqueous media than β 34–42. In fact, peptide β 34–42 appears to have comparable or diminished solubility relative to the amyloid plaque cores (Masters et al., 1985). A recent paper reports the solubilization of cyclosporin in solutions of lithium halides in anhydrous tetrahydrofuran (THF) (Seebach, 1988). We found that peptide β 34–42 is highly soluble (≥ 100 mg/mL) in 2 M LiBr in THF. The soluble entity is presumably a lithium amide carbonyl complex (Seebach et al., 1989). The complex can be broken up and the peptide precipitated by addition of trace amounts of water to the solution.

³ Peptide β 26–33. Amino acid analysis: S, 0.5 (1 expected); N, 1.0 (1); K, 0.7 (1); G, 2.0 (2); A, 0.9 (1); I, 1.6 (2). FABMS: 759.4 (M + H⁺), 781.5 (M + Na⁺); MW = 758.3. ¹H NMR (DMSO, 500 MHz): δ 8.2 (m), 7.93 (d, 1 H, $J = 8$ Hz), 7.88 (d, 1 H, $J = 6$ Hz), 7.65 (m, 1 H), 7.48 (s, 1 H, Asn), 7.02 (s, 1 H, Asn), 4.6 (m, 1 H), 4.33 (pent, 1 H, $J = 8$ Hz, Ala α H), 4.16 (m), 3.7 (m), 2.75 (m, 2 H), 1.7–1.3 (m), 1.2 (d, 3 H, $J = 7.5$ Hz, AlaCH₃), 1.06 (m), 0.8 (m, 12 H).

⁴ Peptide β 34–42. Amino acid analysis: L, 0.9 (1 expected); M, 0.7 (1); V, 2.8 (3); G, 2.1 (2); I, 0.8 (1); A, 1.0 (1). FABMS: 858.4 (M + H⁺); MW = 857.5. ¹H NMR (CF₃CO₂D, 300 MHz): δ 5.05 (m, 1 H), 4.8 (q, 1 H, $J = 6.5$ Hz, Ala α H), 4.6 (m), 4.4 (m), 4.0 (m), 3.85 (m), 2.8 (m), 2.2 (m), 2.1–1.7 (m), 1.65 (d, 3 H, $J = 6.5$ Hz, AlaCH₃), 1.4 (m), 1.05 (m, 30 H).

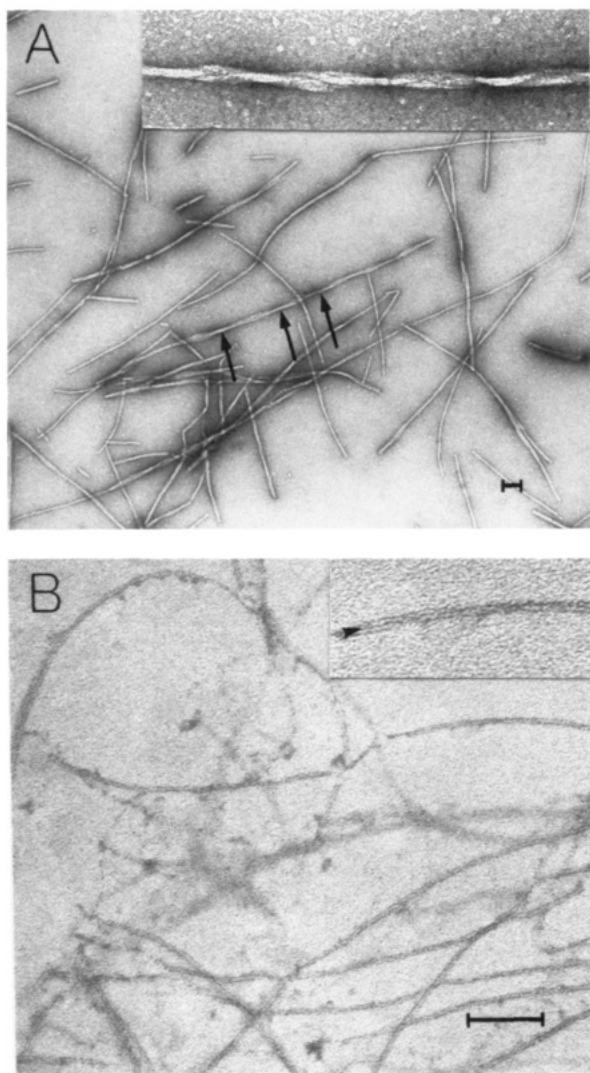


FIGURE 3: Electron micrographs of negatively stained peptide assemblies. (A) Peptide β 34-42 at low magnification shows two types of assemblies: twisted fibers and untwisted narrower fibers. Inset: Higher magnification shows the twisted fibers and the periodic deposition of enhanced staining (arrows). (B) Peptide β 26-33 fibers are thin and uniform compared to those of β 34-42 and appear to be comprised of a pair of fibrils (inset). The scale bars for (A) and (B) are equal to 1000 Å.

Peptide β 34-42 in the Solid State Exists as Antiparallel β -Sheets. The gel-like solid that results from attempts to

dissolve β 34-42 in deuterated water was directly analyzed by FTIR, providing the absorption spectrum shown in Figure 2A. The positions of the amide I band at 1625 cm^{-1} and the weak absorption band at ca. 1700 cm^{-1} are characteristic of antiparallel β -sheet structure (Krimm & Bandekar, 1986). FTIR analysis of solutions of β 26-33 in deuterated water and HFIP and of β 34-42 in HFIP reveal much broader amide I absorptions, centered around 1650 cm^{-1} . This is indicative of a random coil or helical conformation (Krimm & Bandekar, 1986; Doyle et al., 1970). Peptide β 34-42 in 2 M LiBr-THF had a narrower amide I absorption at ca. 1660 cm^{-1} . The position of this band may reflect coordination of lithium at the amide carbonyl oxygen (Seebach et al., 1989). Addition of deuterium oxide to this solution led to formation of a gel that had a spectrum indicative of β -structure (amide I at 1625 cm^{-1} ; see Figure 2B). These experiments demonstrate that precipitation is linked to the formation of β -sheet structure. Peptide β 34-42 exists exclusively as a β -sheet which will not disaggregate in water, whereas peptide β 26-33 is soluble in water as a mixture of non- β -conformers.

Peptides β 26-33 and β 34-42 Form Fibers Having Distinct Morphologies. Peptide β 34-42 formed rigid fibers of varying lengths ranging from 0.01 to 3 μm or greater (Figure 3A). A periodic deposition of stain, which occurred every 0.12–0.14 μm , indicated that the fibers were like twisted ribbons. The resulting axial fluctuation of width was 85–95 Å in the narrowest region where the fiber was uniform and 190–200 Å in diameter in the widest region. The micrographs also showed nontwisted, apparently single fibrils which were slab-like and had a width of 90 Å. The assembly of several of these smaller fibrils likely constitutes the twisted fibers. Examination of β 34-42 under strong denaturing conditions (i.e., 6 M guanidinium thiocyanate, several days at 20 °C) revealed that the insoluble material retained a fibrous morphology but lacked the twisting.

Peptide β 26-23 assembled into very long, thin flexible fibers with a uniform diameter of 50–55 Å (Figure 3B). Each of the negatively stained structures appeared to be comprised of a pair of fibrils wrapped around each other tightly but without a well-defined pitch. The observed morphology suggested that the constituent fibrils were cylindrical rather than slab-like. Occasionally, large bundles of fibers were seen.

Peptides β 26-33 and β 34-42 Form Cross- β -Fibrils. Lyophilized samples of both peptides β 26-33 and β 34-42 showed prominent X-ray reflections at ca. 4.7 and 9–10 Å (Figure 4A) which correspond to the distances between hydrogen-bonded peptide backbones and β -pleated sheets, respectively. Similar

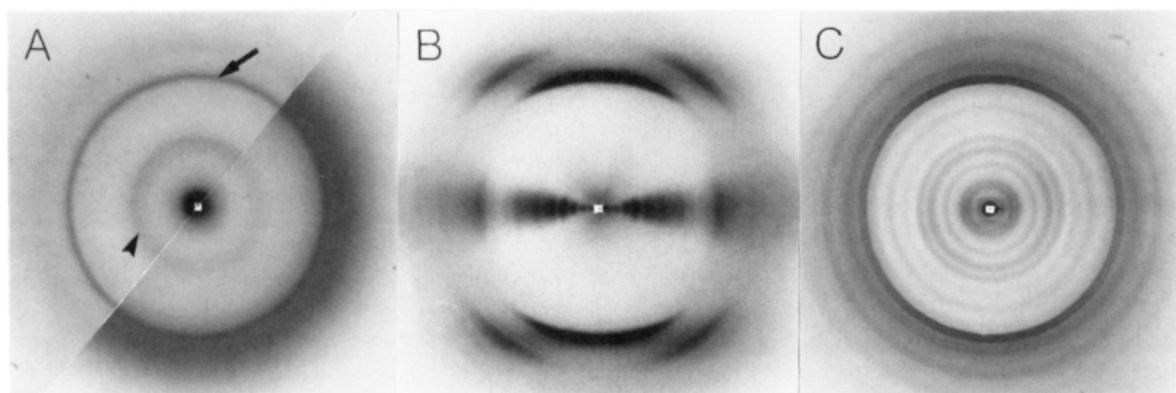


FIGURE 4: X-ray diffraction patterns from the peptides. (A) Lyophilized peptides β 34-42 (upper left) and β 26-33 (lower right). The hydrogen-bond spacing (arrow) and intersheet spacing (arrowhead) are indicated. (B) Pellet of peptide β 34-42 dried from HFIP under wet nitrogen. The most prominent reflection is the hydrogen-bond spacing on the meridian. The apparently large breadth of this spacing is due to superposition of an additional arc at ca. 4.5 Å. (C) Peptide β 26-33 pellet air-dried from 10% formic acid. The fibrils are mostly unoriented; however, the β -pleated sheet spacings are slightly accentuated at right angles to one another.

spacings have been recorded from AD amyloid plaque cores, systemic amyloid, and other synthetic β -amyloid peptides (Termine et al., 1972; van Andel et al., 1986; Kirschner et al., 1986, 1987). During slow evaporation of the peptide β 34–42–HFIP solution in a siliconized capillary, the fibrils sedimented to form a singly oriented pellet, which showed discrete 4.7-Å arcs on the meridian and a series of equatorial spacings (see Figure 4B). The latter include the intersheet spacing at 9–10 Å and spacings coming from intra- and interfiber packing. In the X-ray pattern from peptide β 34–42, in contrast to that of synthetic peptide β 1–28 (Kirschner et al., 1987), there was no detectable meridional 9.4-Å reflection, which would be indicative of antiparallel β -strands. The absence of this reflection is probably due to disorder/disorientation effects in a β -pleated sheet structure which does have antiparallel strands (Fraser et al., 1969). The prominent off-meridional reflection at 3.7 Å has also been observed in X-ray patterns of β 1–28 (Kirschner et al., 1987) and of isolated systemic amyloid (Termine et al., 1972; van Andel et al., 1986).

Preparation of the peptide β 26–33 sample was complicated by its low solubility at high concentration, which resulted in precipitation prior to complete drying. The fibrils nevertheless did orient slightly while sedimenting during drying (Figure 4C). While their X-ray patterns were nearly circularly symmetric, the hydrogen-bond and intersheet spacings were sufficiently accentuated on orthogonal axes to indicate a cross- β -conformation. Many of the other small-angle reflections correspond to oriented spacings observed for β 34–42 and presumably come from the packing of the fibers in the dried samples.

DISCUSSION

We hope to elucidate the molecular basis of aggregation and precipitation of the amyloid β -protein of Alzheimer's disease (AD). Two alternative scenarios for the deposition of amyloid can be imagined, each of which has different implications concerning the behavior of the β -protein. One possibility is that the proteolytic processing of the amyloid precursor is somehow altered in AD, leading to release of an intrinsically insoluble β -protein. Alternatively, the β -protein may be a normal cellular product which precipitates due to extrinsic conditions unique to the disease. Several groups have studied synthetic peptides derived from the N-terminal portion (1–28) of the β -protein (Kirschner et al., 1987; Castaño et al., 1986; Gorevic et al., 1987; Hollosi et al., 1989). Two of these peptides, β 1–28 and β 12–28, form fibrils that ultrastructurally resemble native β -protein. Most of these peptides contain β -structure in the solid state; however, all are water soluble. This paper addresses the intrinsic insolubility of the β -protein through the study of two synthetic peptides derived from its C-terminal sequence. This sequence may be part of the transmembrane domain of APP (Kang et al., 1987). We found that peptide β 34–42 in the solid state exists entirely as antiparallel β -sheet structure, which is extremely resistant to disaggregation and solubilization in aqueous media. We propose that the C-terminal region of the β -protein is a major determinant of its precipitation and may nucleate cross- β -fibril formation.

The peptide β 26–33 was soluble in water, whereas β 34–42 was found to be unusually resistant to solubilization in aqueous media. A correlation between decreased water solubility of a polypeptide and increased formation of β -structure has been noted (Furhop et al., 1987; Przybycien & Bailey, 1989). The β -sheet, while defined by interstrand hydrogen bonding, owes its stability to interstrand and intersheet side-chain packing. The β -branched side chains of valine and isoleucine pack

efficiently along the surface of a β -sheet. In addition, these residues are known to disfavor helical conformations, which are usually water soluble (Arfmann et al., 1977). We found that empirical structural parameters (Chou & Fasman, 1978) were useful as a guideline in predicting the water solubility of these peptides. We propose that soluble peptides of less than 10 residues comprise amino acids which often occur in random coil ($P_c > 1$) or helical structure (Narita et al., 1984, 1988), but are rarely found in β -sheet structure ($P_\alpha - P_\beta > 0$). The soluble peptide β 26–33 met one of these proposed criteria, while β 34–42 did not (see Table I). For larger peptides, amino acid sequence is critical: the clustering of "insoluble" amino acids (e.g., Val and Ile), as it occurs at the C-terminus of the β -protein, can lead to precipitation (note the unusual concentration of β -branched residues: 4 out of 9 residues in β 34–42; 6 out of 12 in the sequence 31–42).

In organic solvents, hydrogen bonding, rather than side-chain packing, drives structure formation (Toniolo et al., 1985; Narita et al., 1988). The failure of β 34–42 to dissolve in most commonly used solvents with the exception of HFIP reflects the reluctance of this sequence to form a soluble helix or coil. The novel solvent system of lithium bromide in anhydrous tetrahydrofuran (Seebach, 1988; Seebach et al., 1989) was shown to be an extremely effective solvent for β 34–42, due to its ability to complex and chelate amides, which are potential hydrogen-bonding partners. The soluble form of β 34–42 contained no β -sheet structure (FTIR; see Figure 2); however, the reappearance of β -structure accompanied precipitation.

Both peptides reported herein, β 26–33 and β 34–42, possessed ordered β -sheet structure in the solid state, as observed by X-ray diffraction. Analysis of the peptide β 34–42 in the solid state by FTIR revealed that the entire peptide sequence is involved in β -sheet structure and that the orientation of the peptides in each sheet is antiparallel. The β 26–33 and β 34–42 fibrils each had distinctive morphologies, and neither resembled the native β -protein fibrils or the similar β 1–28 fibrils (Kirschner et al., 1987). The apparent rigidity of the β 34–42 fibrils, as opposed to the thin, curvilinear nature of the β 26–33 fibrils, may account for the sedimentation and formation of a singly oriented pellet by β 34–42. The oriented fibrils were shown to contain cross- β -structure, as opposed to extended β -structure. The resistance of the β 34–42 fibrils to chemical denaturation suggests an unusually strong interpeptide interaction.

The fact that β 26–33 and β 34–42 possess cross- β -structure in the solid state is not surprising; in fact, this is also the case for many peptides which have helical structure in aqueous solution [e.g., insulin (Glennner et al., 1974)]. However, the unusually low solubility of peptide β 34–42 and the insensitivity of precipitated β 34–42 to chemical denaturation suggest a role for this sequence in amyloid plaque deposition. In addition, it has been reported that the cerebrovascular amyloid (β 1–39/40) is significantly more soluble than amyloid plaque (β 1–42/43) (Prelli et al., 1988; Joachim et al., 1988). With these results in mind, we would like to propose the following scenario for amyloid fibril formation. Strong intermolecular interactions between a sequence comprising at least nine amino acids (34–42) at the C-terminus of the β -protein result in self-association and formation of an antiparallel cross- β fibrillar core. Interactions involving the N-terminal region of the β -protein subsequently lead to elaboration of the characteristic morphology of the fibrils which constitute amyloid plaque. Further elucidation of the intrinsic forces, as well as the important extrinsic factors, which stabilize the β 34–42 fibril is critical to the understanding of amyloid deposition.

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