Models of the β Protein C-Terminus: Differences in Amyloid Structure May Lead to Segregation of "Long" and "Short" Fibrils†

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The Alzheimer's disease (AD) brain is characterized by the presence of extracellular insoluble amyloid plaque, the primary component of which is a *ca.* 4 kDa protein called the β protein.[1] Several variants of the β protein, which differ only at their C-terminus, occur *in vivo*. The predominant circulating form in the blood and cerebrospinal fluid of normal patients, as well as AD patients, is β1–40 or "short β".[2] However, β1–42 and β1–43 ("long βs") are the major variants in AD plaque (Figure 1).[3] The long β proteins form amyloid nuclei much more rapidly than short β protein and are effective seeds for amyloid formation by short β.[4] This kinetic difference suggested to us that production of the long β proteins may be a pathogenic event.[4,5] We examine herein the effect of the C-terminal sequence on the *thermodynamic*[6] stability of the amyloid fibril. Differences in fibril structure were identified indirectly, by measuring the changes in fibril solubility, and directly, by analyzing ^{13}C-labeled fibrils by isotope-edited FTIR[7] and solid-state NMR.[7b] These model studies reinforce the proposal that long β proteins are pathogenic and suggest an explanation for the predominance of long β proteins in AD amyloid plaque.

In contrast to the dramatic differences between short and long model peptides β26–β40 and β26–43 (Figure 1) with respect to their kinetic solubility[8] (the nucleation time of β26–40 is *ca.* 10^4-fold longer[4]), relatively small differences were detected in the thermodynamic solubility (K_g, Figure 2) of the amyloid fibrils (β26–40 is about 8-fold more soluble; β26–40, 5.3 ± 2.6 μM; β26–43, 0.7 ± 0.4 μM).[4,9] The dissolution of β26–40 fibrils (Figure 2) was approximately 10-fold faster than dissolution of β26–43 fibrils.[10]

The solubility differences between the β26–40 and the β26–43 amyloid fibrils are a consequence of subtle structural differences which are reflected in their FTIR spectra (Figure 3, spectrum A *vs* C).[11] Isotope-edited FTIR spectra of β26–40 (Figure 3B) and β26–43 (Figure 3D) fibrils labeled with ^{13}C at the Gly38 amide carbonyl showed that the secondary structure and/or local environment of the Gly38 amide in the two fibrils was completely

β1-43: H_2N-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT-CO_2H
β26-43: Ac-SNKGAIIGLMVGGVVIAT-CO_2H
β1-40: H_2N-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV-CO_2H
β26-40: Ac-SNKGAIIGLMVGGVV-CO_2H

Figure 1. Sequence of the long (β1–43) and short (β1–40) β proteins and the corresponding model peptides used in this study.

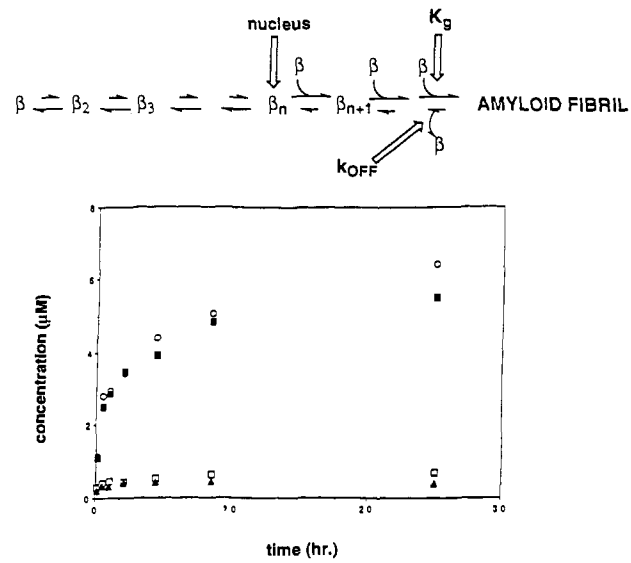

Figure 2. Top: General scheme for nucleation and growth of amyloid fibrils.[17] This study is concerned with the thermodynamic solubility of the amyloid fibril (K_g) and the disaggregation rate of the fibril (k_{OFF}). Bottom: Dissaggregation of amyloid fibrils as measured by radioassay (one typical experiment).[10] The β26–40 fibrils (○) dissolved rapidly to reach a final solubility of *ca.* 6 μM, whereas the β26–43 (□) dissolved more slowly and reached a final solubility of *ca.* 0.5 μM. Fibrils made from seeding β26–40 (■) with β26–43 (▲) (10%) were made with the ^3H radiolabel in either the long (▲) or short (■) component, and the appearance of that component in solution was measured. The seeded fibrils behaved as though the two peptides had formed separate homogeneous fibrils;[15] that is, one peptide does not affect the solubility of the other.

† Dedicated to Peter T. Lansbury, Sr., my first chemistry teacher, on the occasion of his retirement from the Department of Chemistry at the State University of New York at Buffalo.

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(5) The production of β proteins in cell culture was studied. Point mutations in the β amyloid precursor protein which cause early onset AD alter the ratio of β isoforms produced, in favor of the long β isoforms. The total amount of β protein produced was unchanged. Cheung, T. T.; Suzuki, N., Cai, X.-D.; Odaka, A.; Otvos, L.; Eckman, C.; Golde, T. E.; Younkin, S. G. *Science* **1994**, *264*, 1336.

(6) The term thermodynamic is used, although we have not proven that the fibrils discussed herein are not kinetically trapped. However, the fact that the amount of peptide remaining after aggregation[4] and the amount solubilized from preformed fibrils[10] are indistinguishable[9] argues against this possibility.

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(8) Kinetic solubility refers to the metastable supersaturated state which precedes nucleus formation.[4,17]

(9) Thermodynamic solubility: Previous measurements were made by amino acid analysis; thus the error was large.[4] In the present method, tritiated peptides were equilibrated for 1 week in both the forwarded (seeded aggregation)[4] *and* reverse (dissolution)[10] directions, and the solubility was determined by counting the supernatant after filtration (0.22 μm Millipore GV filters). The solubilities obtained in the forward and reverse directions were similar, and the values reported are an average of three measurements in each direction. Peptide synthesis:[4] Peptide-resin was acetylated with [^3H]NaOAc, BOP (2 equiv), and DIEA (4 equiv) in DMF (5 mL/g resin). After cleavage, the crude peptide was stirred in aqueous buffer for 1 week[6] and the resultant tritiated peptide fibrils were collected by centrifugation and characterized by plasma desorption mass spectrometry (80–90% pure).[4] A stock solution of each peptide in hexafluoro-2-propanol (HFIP) was prepared, and the peptide concentration was determined by quantitative amino acid analysis in conjunction with BCA assay.[4] The specific activity of the peptides prepared by this method was typically *ca.* 10,000 cpm/nmol.

(10) Amyloid dissolution: Dissolution is proportional to the disaggregation rate constant (k_{OFF}). ^3H-labeled peptide fibrils[4] (60 nmol) were collected by centrifugation (3000*g*), and the supernatant was decanted. Fresh buffer (3 mL, 100 mM NaCl, 10 mM NaH_2PO_4, pH 7.4) was added and the concentration of soluble peptide followed by removing and filtering ~100 μL of the suspension (0.22 μm Millipore GV filters), followed by scintillation counting of the filtrate. Initial dissolution velocities under these conditions: β26–40, 6×10^{-4} μM/s; β26–43, 6×10^{-5} μM/s.

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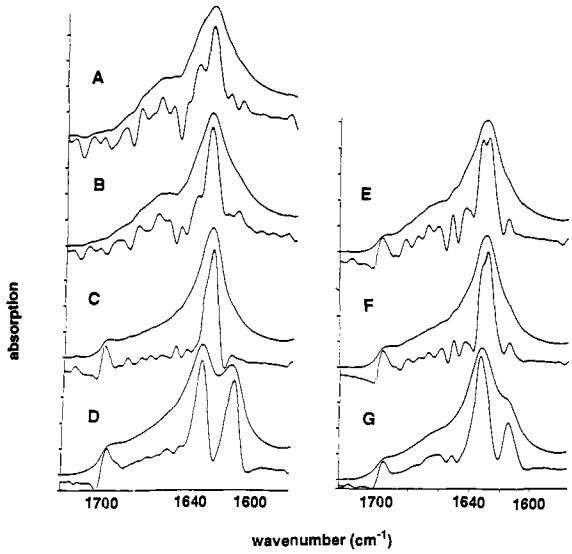

Figure 3. Isotope-edited FTIR analysis of peptide amyloid fibrils. The raw absorption data is overlayed with the second-derivative spectrum, which was used to determine peak position.[13] All spectra were normalized for this figure. Left panel: Infrared spectra (amide I region, predominant peak positions listed) of β26–40 and β26–43 amyloid fibrils. A: Unlabeled β26–40 (1629 cm^{-1}). B: β26–40 [^{13}C]Gly38 carbonyl (1630). C: Unlabeled β26–43 (1628, 1698). D: β26–43 [^{13}C]Gly38 carbonyl (1613, 1634, 1695). Right panel: spectra of fibrils formed under seeding conditions (all *ca.* 90% β26–40).[16] E: Both peptides unlabeled (1632/1628). F: β26–40 labeled (1629). G: β26–43 seed labeled (1614, 1632).

different. The ^{13}C amide absorption (1613 cm^{-1}) in the β26–43 spectrum (Figure 3D,[11] is absent in the β26–40 spectrum (Figure 3B)).[12,13] In addition, introduction of the ^{13}C amide into the β26–43 fibrils (Figure 3C to Figure 3D) caused a shift in the ^{12}C amide bands (1628 cm^{-1} to 1635 cm^{-1} and 1698 cm^{-1} to 1695 cm^{-1}) which indicated disruption of transition dipole coupling, a signature of antiparallel β sheet structure.[7a,b] Labeling of β26–40 (Figure 3A to Figure 3B) did not produce an analogous shift. The structural difference between the β26–40 and β26–43 amyloid fibrils may involve intermolecular orientation rather than the peptide backbone, since solid-state ^{13}C NMR analysis of the two labeled fibrils suggested that the backbone conformation at Gly37-Gly38 was unchanged, although this region may be less ordered in the β26–40 fibril.[14]

Despite the structural differences between the two peptide fibrils, integrated fibrils could possibly be formed under conditions where β26–43 seeds amyloid formation by β26–40.[4] Heterogeneous, or integrated, fibrils would be expected to have properties which differ from those of the two homogeneous, or segregated, fibrils.[15] Alternatively, integrated fibrils may be transient kinetic intermediates which are responsible for seeding,[4] but eventually give way to more stable homogeneous fibrils. In that case, the properties of the fibrils formed by seeding would be equal to a weighted sum of the properties of the two homogeneous fibrils. The experimental approaches discussed above were used to distinguish between these two scenarios.

In two otherwise identical experiments, fibrils were formed by seeding β26–40 with β26–43; a radiolabel was contained in either the short or the long (seed) peptide.[4,16] With respect to their rate of dissolution[10] and their final solubility,[9] the "seeded" fibrils behaved as though separately formed, homogeneous fibrils had been combined (Figure 2). Examination of the seeded fibrils by traditional FTIR (Figure 3, spectrum E) could not distinguish between the two alternatives discussed above. However, isotope-edited FTIR (Figure 3F,G) supported the notion that more stable, segregated fibrils are produced by seeding. Again, fibrils were formed in which either the major component (β26–40, Figure 3F) or the seed peptide (β26–43, Figure 3G) was labeled, this time with ^{13}C at Gly38.[16] In both cases, the resultant spectra are indistinguishable from the weighted sum of the "pure" spectra (i.e., 3F = 0.9(3B) + 0.1(3C) and 3G = 0.9(3A) + 0.1(3D)). The FTIR data and the solubility measurements are consistent with the notion that segregated fibrils are formed from heterogeneous seeding.[15]

Thermodynamic differences between the long (β1–42 or β1–43) and short (β1–40) β protein homogeneous amyloid fibrils may have significant consequences for AD pathogenesis.[4,5] First, the critical concentration for amyloid formation by short β is greater than that for long β; hence one could bear a greater load of soluble short β without amyloidogenesis occurring. Second, short β amyloid, once formed, could dissolve more rapidly than long β amyloid, making its clearance feasible. This would explain the apparent "concentration" of long β in AD amyloid plaque.[3] Thus, the total amount of long β proteins produced, regardless of the amount of short β, may be the critical factor for the induction of AD pathogenesis.[4,5]

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(11) The β26–40 fibrils (Figure 3A) may contain parallel, rather than antiparallel, β sheet, as indicated by the lack of the diagnostic high-frequency absorption at *ca.* 1698 cm^{-1} (see Figure 3C). The large intensity of the ^{13}C Gly38 amide absorption (1613 cm^{-1}, Figure 3D) relative to the ^{12}C absorption (1634) is also typical of antiparallel β sheet structure.[7a,b]

(12) Synthetic peptides[4] were characterized by PDMS (>90% pure). These peptides were labeled with ^{13}C at the α carbon of Gly37 and the carbonyl carbon of Gly38. Labeling at the α carbon did not perturb the amide I absorption band.[7] Amyloid fibrils for infrared spectroscopy and solid-state NMR were prepared by dissolving peptide in DMSO and adding to aqueous buffer (200–300 μM peptide, 5% DMSO in buffer). Peptide fibrils were collected by centrifugation and dried *in vacuo*.

(13) Amyloid fibrils[12] were spread on a CaF_2 plate and dried. Excess salt was removed by rinsing with H_2O and drying *in vacuo*. Spectra were recorded on a Perkin-Elmer 1600 series spectrophotometer: 16 scans were averaged at 2 cm^{-1} resolution, the background due to air was subtracted, and the spectra were smoothed with an 8 cm^{-1} smoothing function. Peak positions were determined with the aid of second-derivative analysis (Figure 3).

(14) Reported chemical shifts in parts per million (and line widths in hertz; ^{13}C frequency = 100 MHz ±0.1 ppm) for β34–42: Gly37 α carbon, 45 ppm (290); Gly38 carbonyl carbon, 170 ppm (190).[18] Measured SSNMR data: β26–40, 44 (420), 170 (370); β26–43, 44 (300), 169 (180).

(15) Integrated fibrils would involve both peptides (the ratio need not be 1:1), evenly distributed throughout. Segregated fibrils may be completely separate, homogeneous fibrils or may involve homogeneous regions within the same fibril.

(16) Seeding of fibril formation could be accomplished by adding preformed seed fibrils (*ca.* 10% by moles) to a supersaturated solution or by coaggregation of a peptide mixture.[4] The seeded FTIR spectra (Figure 3E–G) do not depend on the method of seeding.

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