A Conformation Change in the Carboxyl Terminus of Alzheimer’s Aβ(1–40) Accompanies the Transition from Dimer to Fibril as Revealed by Fluorescence Quenching Analysis*

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Alzheimer’s disease is characterized by the presence of insoluble, fibrous deposits composed principally of amyloid β (Aβ) peptide. A number of studies have provided information on the fibril structure and on the factors affecting fiber formation, but the details of the fibril structure are not known. We used fluorescence quenching to investigate the solvent accessibility and surface charge of the soluble Aβ(1–40) dimer and amyloid fibrils. Analogs of Aβ(1–40) containing a single tryptophan were synthesized by substituting residues at positions 4, 10, 34, and 40 with tryptophan. Quenching measurements in the dimeric state indicate that the amino-terminal analogs (AβF4W and AβY10W) are accessible to polar quenchers, and the more carboxy-terminal analog AβV34W is less accessible. AβV40W, on the other hand, exhibits a low degree of quenching, indicating that this residue is highly shielded from the solvent in the dimeric state. Correcting for the effect of reduced translational and rotational diffusion, fibril formation was associated with a selective increase in solvent exposure of residues 34 and 40, suggesting that a conformation change may take place in the carboxy-terminal region coincident with the dimer to fibril transition.

Alzheimer’s disease is a progressive neurodegenerative disease that is characterized by the abnormal accumulation of β-amyloid in senile plaques. Biochemical analysis of the amyloid peptides isolated from Alzheimer’s disease brain indicates that amyloid β (Aβ) (1–42) is the principal species associated with senile plaque amyloid (1), whereas Aβ(1–40) is more abundant in a soluble form in cerebrospinal fluid. Synthetic Aβ(1–40) is relatively soluble, and its aggregation and assembly is a dynamic process, with a number of factors affecting the rate and equilibrium of fibril assembly (2). Key parameters promoting the assembly of amyloid fibril and sedimentable aggregates include high peptide concentration, long incubation times, low pH (pH 5–6), and mechanical agitation (3–8). The length of the carboxyl terminus is also critical in determining the assembly dynamics. The longer Aβ(1–42) isoform aggregates more rapidly at pH 7.4 (4, 7). These observations suggest that aggregation of Aβ may be a critical event in pathogenesis.

The amyloid fibril was shown to be a β-pleated sheet structure using x-ray diffraction (6). These studies established that the amyloid fibril is made of an orthogonal lattice of β-crystallites having unit dimensions of 9.4 Å in a hydrogen bond direction, 7 Å in the polypeptide backbone direction, and 10 Å inter-sheet spacing, arranged in a cylindrical fashion (6). The peptide is organized in a cross β pattern in which the hydrogen bonding direction is parallel to the fiber axis. The Aβ(1–40) sequence is divided in two regions. Residues 1–28 make up a relatively hydrophilic domain with a high proportion of charged residues (46%). In the amyloid precursor protein, this domain is extracellular. The carboxyl-terminal 28–40 residues make up a richly hydrophobic domain that is associated with the cell membrane in the amyloid precursor protein (2). Replacement of hydrophobic residues by hydrophilic residues markedly stabilizes Aβ peptides against aggregation (8), whereas replacement of hydrophilic residues by hydrophobic residues alters the morphology of the fibril, suggesting that hydrophilic residues are largely responsible for the specificity of intermolecular interaction within the fibril (8).

Amyloid fibril formation may involve two basic steps, the initial nucleation of aggregates that establishes the amyloid fibril lattice (6), followed by the elongation of the fibril by the sequential addition of subunits (9). Previous studies indicate that Aβ(1–40) forms stable dimers in solution (10–13) and suggest that dimerization is the initial event in amyloid aggregation and that the dimer represents the fundamental building block for further fibril assembly. However, much remains to be elucidated regarding the structure of the amyloid fibril and the mechanism of amyloid dimer-fibril transition. In this work, fluorescence spectroscopy was used to assess solvent exposure and surface charge around discrete sites along the peptidyl backbone of Aβ(1–40) in both the dimeric and fibrillar states. Specifically, a functional set of single-residue Trp replacement analogs of Aβ(1–40) was synthesized, and the capacity of cationic Cs⁺, anionic I⁻, and neutral acrylamide to dynamically quench the emission from the substituted Trps in these analogs was measured (20). Determining which parts of Aβ are accessible to the solvent and how these environments change during polymerization will be important to understand
how the peptide is organized within the fiber and to identify critical contact sites that are necessary for higher order assembly into fibrils.

**EXPERIMENTAL PROCEDURES**

**Materials**—All peptides were synthesized by fluoranyl-9-ylmethoxy-carbonyl chemistry using a continuous flow semiautomatic instrument as described previously (4). A single tryptophan residue was substituted into the sequence of the Aβ(1–40) peptide at positions 4 (AβF4W), 10 (AβY10W), 34 (AβL34W), and 40 (AβY40W). The peptides were purified by reverse-phase high-performance liquid chromatography. The purity and expected structure was verified by electrospray mass spectrometry. Only peptides exhibiting 90.0% or greater purity with less than 5.0% of a single contaminant were used. All other reagents were of the highest analytical grade commercially available.

**Aggregation Measurements**—Aggregation was determined by using a sedimentation assay as described previously (4, 11). Aβ(1–40) (75 μM) was mixed with the tryptophan analogs of Aβ(1–40) (5 μM) in either 0.1 M NaAc, pH 5.0, 0.1 M NaCl, 20 mM Tris-HCl, pH 7.4, or 0.1 M NaCl, 20 mM Tris-HCl (70 μM ZnCl₂) and incubated for 48 h at room temperature. The samples were centrifuged at 15,000 × g for 10 min. Afterward, the amount of fluorescence in both the supernatant and pellet was determined by measuring the intensity of tyrosine fluorescence for wild type Aβ(1–40) (λ_em = 280 nm and λ_exc = 310 nm) or tryptophan fluorescence for Trp-analogs (λ_em = 295 nm and λ_exc = 350 nm).

**Gel Filtration Chromatography**—Gel filtration analysis was performed with an Amersham Pharmacia Biotech Superdex 75 HR 10/30 column. The peptides were detected by UV absorbance at 280 nm. The mobile phase was 50 mM Tris-HCl/0.1 mM NaCl, pH 7.4 (Buffer A) with a flow rate of 0.4 ml/min. The standards used to calibrate the column were thyroglobulin (670 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa), soybean trypsin inhibitor (23 kDa), ubiquitin (8.5 kDa), and acetone (0.058 kDa).

**Fibril Formation Assays**—Fibril formation was monitored using a thioflavin T (ThT) fluorescence assay (14, 15). The peptides (230 μM) were incubated for 24 h at room temperature in 50 mM Tris, 0.1 M NaCl, pH 7.4, with continuous stirring. After 24 h, 10-μl aliquots of each sample were transferred to a cuvette containing 2 ml of 3 mM ThT, pH 7.4. The fluorescence emission was monitored at 482 nm with excitation at 450 nm using a Spex Fluorolog-2 spectrofluorometer. For electron microscopy analysis, the peptides were incubated for 24 h at room temperature with continuous stirring, at a concentration of 230 μM in 10 mM MOPS, pH 7.4. A drop of each sample was placed on a carbon coated copper grid, negatively stained with 2% aqueous uranyl acetate, and visualized with a Zeiss 10CR microscope (80 kV).

**Fluorescence Quenching Experiments**—Steady-state fluorescence quenching experiments were performed using two different conditions: with the dimeric and fibrillar peptides. For the dimer, aliquots of the stock quenching solutions (5 μM) were added into a 0.5 × 0.5-cm cuvette containing 10 μM of the peptide in Buffer A (0.1 M NaCl, 20 mM Tris, pH 7.4). For the fibrillar state measurements, the peptides (230 μM) were previously incubated for 24 h in Buffer A with continuous stirring. Steady-state quenching experiments were performed with excitation at 295 nm, emission at 350 nm. Corrections were made for dilution. Stock quenching solutions of KI, acrylamide, and CsCl were freshly prepared at 5 μM. Quenching data were fit to the Stern-Volmer equation,

\[
F/F = 1 + K_{SV}[Q]
\]

(Eq. 1)

where \(F\) and \(F_0\) are the fluorescence intensity in the absence and in the presence of quencher \([Q]\), respectively, \(K_{SV}\) is the Stern-Volmer quenching constant. The apparent bimolecular quenching rate constant, \(k_q\), a measure of solute accessibility, was calculated with the following expression,

\[
k_q = K_{SV}/(r)
\]

(Eq. 2)

where \(<r>\) is the geometric average fluorescence lifetime obtained from time-resolved measurements (16, 17).

**RESULTS**

**Aggregation and Fibril Forming Properties of Aβ(1–40) Tryptophan Analogs**—To validate the utility of the quenching experiments employing Aβ substitution analogs, the aggregation properties of the Trp substitution analogs were characterized. Aβ(1–40) was chosen for these studies because its dimeric state is stable over the time frame of the experiments and the transition to the fibrillar state can be accomplished by stirring the peptide for 24 h under the same physiological conditions (10–13).

The oligomeric assembly state of the amyloid fibril-forming Aβ(1–40) was characterized by gel filtration chromatography. The peptides eluted at the same position as wild type Aβ(1–40) (Fig. 1). The elution position corresponded to an apparent molecular mass of 9 kDa, determined by the elution behavior of a series of calibration standards as reported previously (8, 10–13) (Fig. 1, inset). The other tryptophan analogs used in this study also eluted as a dimer (data not shown).

Aggregation was determined using a sedimentation assay as described under “Experimental Procedures.” The aggregation properties of the tryptophan analogs and wild type Aβ(1–40) are shown in Fig. 2, under several conditions that are known to modulate the assembly state of Aβ. At pH 7.4, in the presence or absence of zinc ions, and at pH 5.0, the sedimentation behavior of all of the tryptophan analogs used is comparable to wild type Aβ(1–40). Several other substitution analogs were synthesized, but they exhibited significantly altered aggregation properties and were not studied further (data not shown).

Thioflavin T binding assays were also used to follow the progress of amyloid fibril formation for the tryptophan analogs. In comparison to the wild type Aβ(1–40), all the tryptophan analogs were able to bind thioflavin T as judged by the high fluorescence, but AβY10W showed a significantly lower fluorescence (Fig. 3). To further verify the fibril forming properties of the tryptophan analogs, their structures were examined by electron microscopy. Electron microscopy indicated that the tryptophan analogs AβY10W and AβL34W form fibrils that are
The ThT binding of all the tryptophan analogs and wild type Aβ(1–40) were compared under physiological conditions in which the peptide behaves as a dimer (Tris-HCl buffer, pH 7.4) and under two conditions that promote fibrillization: at pH 5.0 and at pH 7.4 in the presence of 70 μM ZnCl₂. Aβ(1–40) (75 μM) was mixed with 5 μM of fluorescent Trp Aβ(1–40) analog as described under “Experimental Procedures” and incubated for 48 h at room temperature. The samples were centrifuged at 15000 × g for 10 min. Afterward, the amounts of fluorescent Trp analog and wild type Aβ(1–40) in both the supernatant and the pellet were determined by measuring the fluorescence intensity.

The aggregation properties of the tryptophan analogs and wild type Aβ(1–40) tryptophan analogs was assessed by employing Thioflavin T binding of tryptophan derivatives of Aβ(1–40). The ThT binding of all the tryptophan analogs and wild type Aβ(1–40) are compared. The peptides were incubated for 24 h at room temperature in 50 mM Tris-HCl, 0.1 M NaCl, pH 7.4, at 1 mg/ml (230 μM) with continuous stirring. After 24 h, 10-μl aliquots of each sample were transferred into a cuvette containing 3 ml of 3 mM ThT, pH 7.4, and the fluorescence was measured at 482 nm upon excitation at 450 nm.

Fig. 2. Aggregation properties of Aβ(1–40) Trp analogs. The aggregation properties of the tryptophan analogs and wild type Aβ(1–40) were compared under physiological conditions in which the peptide behaves as a dimer (Tris-HCl buffer, pH 7.4) and under two conditions that promote fibrillization: at pH 5.0 and at pH 7.4 in the presence of 70 μM ZnCl₂.

Fig. 3. Thioflavin T binding of tryptophan derivatives of Aβ(1–40). The ThT binding of all the tryptophan analogs and wild type Aβ(1–40) are compared. The peptides were incubated for 24 h at room temperature in 50 mM Tris-HCl, 0.1 M NaCl, pH 7.4, at 1 mg/ml (230 μM) with continuous stirring. After 24 h, 10-μl aliquots of each sample were transferred into a cuvette containing 3 mM ThT, pH 7.4, and the fluorescence was measured at 482 nm upon excitation at 450 nm.

The surface charge surrounding the tryptophan residues in the Aβ(1–40) tryptophan analogs was assessed by employing transmission electron microscopy. Negatively stained fibrils formed by all Aβ(1–40) tryptophan analogs exhibited similar morphology to wild type under electron microscopy. Representative fibrils from AβY10W (A) and AβL34W (B) are shown here.
Conformation Change in Aβ(1–40)

Fluorescence quenching parameters for dimeric and fibrillar Aβ(1–40) tryptophan analogs

| λem (nm) | τ (ns) | Acrylamide | KSV (M⁻¹) | kq (s⁻¹) | KI | kq (s⁻¹) | CsCl | kq (s⁻¹) |
|----------|--------|------------|------------|---------|----|---------|------|---------|
| Dimer    |        |            |            |         |    |         |      |         |
| AβF4W    | 350    | 2.1        | 7.2 ± 0.1  | 3.5 ± 0.2 |     | 3.3 ± 0.06 | 1.6 ± 0.1 | 1.1 ± 0.05 | 0.5 ± 0.1 |
| AβY10W   | 350    | 2.3        | 7.6 ± 0.09 | 3.3 ± 0.2 |     | 2.3 ± 0.13 | 1.0 ± 0.1 | 1.2 ± 0.1  | 0.52 ± 0.2 |
| AβL34W   | 350    | 2.0        | 4.7 ± 0.14 | 2.4 ± 0.2 |     | 1.6 ± 0.1  | 0.8 ± 0.1 | 0.7 ± 0.05 | 0.35 ± 0.2 |
| AβV40W   | 346    | 3.0        | 1.9 ± 0.07 | 0.62 ± 0.05 | | 1.2 ± 0.05 | 0.36 ± 0.05 | 0.4 ± 0.03 | 0.13 ± 0.04 |
| Fiber    |        |            |            |         |    |         |      |         |
| AβF4W    | 348    | 2.1        | 3.8 ± 0.12 | 1.8 ± 0.2 |     | 0.6 ± 0.07 | 0.28 ± 0.03 | 1.2 ± 0.04 | 0.57 ± 0.1 |
| AβY10W   | 348    | 1.6        | 2.4 ± 0.02 | 1.5 ± 0.1 |     | 6.0 ± 0.17 | 3.8 ± 0.4  | 1.4 ± 0.09 | 0.89 ± 0.2 |
| AβL34W   | 348    | 1.4        | 4.3 ± 0.09 | 3.1 ± 0.3 |     | 3.2 ± 0.15 | 2.3 ± 0.3  | 0.6 ± 0.09 | 0.42 ± 0.2 |
| AβV40W   | 346    | 1.5        | 5.0 ± 0.18 | 3.3 ± 0.4 |     | 0.6 ± 0.04 | 0.39 ± 0.1 | 0.4 ± 0.07 | 0.026 ± 0.04 |

veh AβY4W should have been associated with a kq value of ~0.8 nM⁻¹ s⁻¹, whereas the observed value was significantly lower, 0.3 nM⁻¹ s⁻¹, suggesting a small fibrillation formation-induced increase in the negative surface charge near position 4. Similarly, if only translational and rotational diffusion rates changed upon fibril formation, then the kq values for the AβY10W, AβL34W, and AβL40W should have been 0.8, 0.5, and 0.2 nM⁻¹ s⁻¹, respectively. However, the kq values were observed to be significantly higher, 3.8, 2.3, and 0.4 nM⁻¹ s⁻¹, respectively (Table I and Fig. 6), suggesting an increase in the positive surface charge near positions 10, 34, and 40 following fibril formation.

Although the kq values for cesium quenching of the dimeric analogs are 80–86% less than the respective kq values for acrylamide quenching, their rank order is the essentially same as that of acrylamide (AβY4W = AβY10W > AβL34W > AβL40W) (Table I). This suggests that the surface charge about each substituted tryptophan has little effect on cesium quenching of the dimeric analogs and that cesium is intrinsically a less efficient tryptophan quencher than acrylamide.

Fibril formation was associated with differential effects on the capacity of cesium to quench the emission from the substituted tryptophan analogs. Assuming that there were no changes in the surface charge upon fibril formation and that the only changes in quenching are due to reduced translational and rotational diffusion (31), fibrillar AβY4W should have been about 0.25, 0.26, 0.18, and 0.07 nM⁻¹ s⁻¹ for AβY4W, AβY10W, AβL34W, and AβL40W, respectively. However, the observed kq values for the AβY4W, AβY10W, and AβL34W analogs were 2.3–3.4 times higher and 2.5 times lower for the AβL40W analog that what would be expected if there were changes in surface charge around the substituted tryptophans.

indicative of partially exposed tryptophans (20). The relative susceptibility of the substituted tryptophans in the dimeric peptides to iodide quenching is similar to that observed with acrylamide and suggests that surface charge has little effect on the iodide quenching (Table I and Fig. 6).

The formation of fibrils was associated with differential changes in the surface charge adjacent to the substituted tryptophans. Correcting for the effect of reduced translational and rotational diffusion (31), fibrillar AβY4W should have been associated with a kq value of ~0.8 nM⁻¹ s⁻¹, whereas the observed value was significantly lower, 0.3 nM⁻¹ s⁻¹, suggesting a small fibrillation formation-induced increase in the negative surface charge near position 4. Similarly, if only translational and rotational diffusion rates changed upon fibril formation, then the kq values for the AβY10W, AβL34W, and AβL40W should have been 0.8, 0.5, and 0.2 nM⁻¹ s⁻¹, respectively. However, the kq values were observed to be significantly higher, 3.8, 2.3, and 0.4 nM⁻¹ s⁻¹, respectively (Table I and Fig. 6), suggesting an increase in the positive surface charge near positions 10, 34, and 40 following fibril formation.

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upon fibril formation (Table I and Fig. 6). This suggests that fibril formation is associated with an increased negative charge around positions 4, 10, and 34 and an increase in positive charge around position 40. These results are only partially consistent with the iodide quenching results. Whereas both the iodide and cesium results indicated increased negative surface charge around position 4 and increased negative charge around position 40, the iodide suggested increased positive and the cesium increased negative surface charge around positions 10 and 34. Collectively, the cesium and iodide quenching results would indicate that fibril formation was associated with a homogeneous increases in negative surface charge around position 4 and positive surface charge around position 40 but inhomogeneous increase in both positive and negative charge around positions 10 and 34.

**DISCUSSION**

The primary objective of the present work was to map the solvent-accessible surface of Aβ(1–40) in the dimeric and fibrillar states by using fluorescent quenching analysis. The aromatic amino acids that are present at positions 4 and 10 (Phe and Tyr) of Aβ(1–40) were substituted with a tryptophan. Hydrophobic carboxyl-terminal residues 34 and 40 (Met and Val) were also substituted with tryptophan. Substitution of the other aromatic residues at positions 19 and 20 significantly inhibited the aggregation of the peptides and were not studied further. This general strategy has been used to study the structure and function of proteins and peptides from which the Trp aromatic amino acid is absent (21, 23–26). This approach was recently used (27) to study ligand dependent changes in the accessibility of P-glycoprotein induced by different antimtumor agents by using quenching analysis. They found that upon addition of the substrate, the enzyme adopts a different tertiary structure, resulting in a significantly increased solvent accessibility.

The single tryptophan analogs examined in this work can be classified into two groups on the basis of their sensitivity to collisional quenchers: exposed and minimally exposed. Tryptophan residues in dimeric Aβ40, Aβ110W, and Aβ134W are exposed and display high bimolecular quenching rate constants with acrylamide and iodide (Fig. 6). Comparison of the degree of quenching of these residues with iodide and cesium indicates that in the dimeric state, residues 4 and 10 are surrounded by positively and negatively charged amino acids. This is in good agreement with the published model of the Aβ dimer (22), in which the negatively charged residues (Asp-1, Glu-3, Asp-7, Glu-11, and Asp-23) are on the surface of the molecule, and the basic residues (His-13, His-14, and Lys-16) represent a positively charged patch site. Residues 10 and 34 are surrounded by both positive and negative charges, position 4 by primarily negative charges, and position 40 by positive charges. The tryptophan residue at position 40 is highly exposed to the solvent in the fibrillar state but is significantly less exposed in the dimeric state (Fig. 6).

The measurements described in this paper clearly suggest that this change in exposure is indicative of a conformational change that occurs in this region accompanying the dimer to fibril transition, which may be an important step in amyloid assembly. The existence of such a conformational change could be due to the involvement of distinct peptide domains in the association of amyloid dimers, which eventually lead to higher order aggregates and subsequent fibril formation.

This observation of a conformation change is consistent with previous studies of the temperature dependence of fibril formation, which suggested such a conformational change on the basis of the unusually high activation energy for the transition (29). Although these studies were conducted in 0.1 M HCl, we also find evidence of a conformational change under more physiological conditions. Our data further indicate that this conformation change takes place at the carboxyl terminus. This conformation change may confound attempts to elucidate the structure of the Aβ peptide in amyloid fibrils, because most of the molecular models have assumed that no change in conformation takes place after initial dimerization (22, 30).

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