Fibrillogenesis in Alzheimer's disease of amyloid β peptides and apolipoprotein E

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A central event in Alzheimer's disease is the conformational change from normally circulating soluble amyloid β peptides (Aβ) and tau proteins into amyloid fibrils, in the form of senile plaques and neurofibrillary tangles respectively. The apolipoprotein E (apoE) gene locus has recently been associated with late-onset Alzheimer's disease. It is not known whether apoE plays a direct role in the pathogenesis of the disease. In the present work we have investigated whether apoE can affect the known spontaneous *in vitro* formation of amyloid-like fibrils by synthetic Aβ analogues using a thioflavine-T assay for fibril formation, electron microscopy and Congo Red staining. Our results show that, under the conditions used, apoE directly promotes amyloid fibril formation, increasing both the rate of fibrillogenesis and the total amount of amyloid formed. ApoE accelerated fibril formation of both wild-type Aβ-(1–40) and Aβ-(1–40A), an analogue created by the replacement of valine with alanine at residue 18, which alone produces few amyloid-like fibrils. However, apoE produced only a minimal effect on Aβ-(1–40Q), found in the Dutch variant of Alzheimer's disease. When recombinant apoE isoforms were used, apoE4 was more efficient than apoE3 at enhancing amyloid formation. These *in vitro* observations support the hypothesis that apoE acts as a pathological chaperone, promoting the β-pleated-sheet conformation of soluble Aβ into amyloid fibres, and provide a possible explanation for the association of the apoE4 genetic isoform with Alzheimer's disease.

INTRODUCTION

Accumulation of aggregated amyloid β-peptides (Aβ) is one of the major biochemical abnormalities found in Alzheimer's disease senile plaques and cerebral blood vessels. Aβ is a 39–44-residue peptide [1–6] derived from normal internal proteolysis of a group of larger (100–140 kDa) ubiquitous membrane-associated glycoproteins known as β precursor proteins. Soluble species of Aβ essentially identical in their primary structure with those found in amyloid deposits are present at low concentrations in plasma and cerebrospinal fluid from both normal individuals and patients with Alzheimer's disease [7–10]. The mechanisms by which soluble Aβ deposition is initiated in the diseased brain are unknown. In certain hereditary forms of early-onset Alzheimer's disease, mis-sense mutations resulting in amino acid substitutions in the Aβ sequence have been directly implicated in the pathogenesis of the disease, either leading to overproduction of Aβ [11,12] or increasing its tendency to self-aggregate. The latter mechanism has been demonstrated to occur *in vitro* with a synthetic analogue of Aβ containing the Gln-22 substitution characteristic of the Dutch variant of cerebral amyloid angiopathy, a rare vascular form of familial Alzheimer's disease [13–16]. Yet these hereditary forms of the disease account for a small proportion of cases, and the molecular pathogenesis in the vast majority of late-onset sporadic cases remains obscure.

Recent genetic studies suggest a close association between one of the main isoforms of apolipoprotein E (apoE) encoded by the ε4 allele and both familial and sporadic late-onset Alzheimer's disease [17,18]. A higher incidence of the ε4 allele in these two groups of patients compared with the normal age-matched population has been reported. The inheritance of the ε4 allele has been associated with an earlier age of onset [17] and a greater number of senile plaques and Aβ immunoreactivity, following an apparent allele–dose effect [19]. *In vitro* studies have shown that apoE can bind to synthetic Aβ [18,20] and tau protein [21], the main constituent of paired helical filaments (PHF) in neurofibrillary tangles [22]. Together these data have led to the hypothesis that apoE plays a central pathogenetic role in Alzheimer's disease. However, apoE immunoreactivity is present in other types of cerebral amyloidosis such as Down's syndrome (related to Aβ), cystatin C-related Icelandic-type hereditary amyloid angiopathy and the prion-associated amyloid of Creutzfeldt–Jakob disease [23,24]. In addition, apoE immunoreactivity is a consistent finding in all types of systemic amyloidoses, and apoE fragments have been copurified with amyloid A subunits from cases of familial Mediterranean fever [23,25]. This widespread association of apoE with biochemically diverse amyloids has led us to postulate a more general role for it in the process of amyloid formation. This concept implies that apoE may modify and/or promote the aggregation of amyloidogenic proteins into the β-sheets that typically constitute amyloid by nucleating fibril growth. The purpose of the present study was to test this hypothesis *in vitro* using, as a model system, synthetic peptides analogous to Aβ by means of a thioflavine-T (Th-T) fluorescent assay [26,27], electron microscopy and Congo Red staining.

EXPERIMENTAL

Synthetic peptides and proteins

Peptides Aβ-(1–40) and Aβ-(1–40Q) corresponding to residues 1–40 and 1–40 containing a Glu–Gln substitution at position 22

Abbreviations used: Aβ, amyloid β peptide; apoE, apolipoprotein E; Th-T, thioflavine-T; PHF, paired helical filaments.

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of Aβ respectively were synthesized by solid-phase procedures at the Center for Analysis and Synthesis of Macromolecules (SUNY, Stony Brook, NY, U.S.A.). The peptide containing a Val→Ala replacement at position 18 was synthesized as previously indicated [28]. Crude peptides were purified by h.p.l.c. using a μ-Bondapak C18 column (3.8 mm × 300 mm) and a linear gradient of 0–80% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 2 ml/min. Peptide sequences were determined by automatic Edman degradation on a 477A protein sequencer and the phenylthiohydantoin derivatives analysed with an on-line 120A phenylthiohydantoin analyser (Applied Biosystems). Estimation of purity and quantification of the peptides was carried out by amino acid analysis using a Pico-Tag analyser (Waters). Human apoE was purchased from Cortex Biochem, and its purity (> 95%) was assessed by SDS/PAGE and N-terminal sequence analysis as described above. Human recombinant apoE isoforms produced in baculovirus were purchased from PanVera Corporation (Madison, WI, U.S.A.). Bovine ubiquitin and BSA were obtained from Sigma. Protein content of these preparations was determined using a micro bicinchoninic acid protein assay reagent kit (Pierce).

Aggregation and fluorimetric experiments

Stock solutions of peptides were prepared in 0.1% trifluoroacetic acid/50% acetonitrile and stored at −20 °C; peptide concentration was determined by amino acid analysis. Portions of this stock were lyophilized and incubations were started by adding the appropriate buffers or cerebrospinal fluid which had been ultrafiltered on a 1 kDa cut-off membrane (Omega cell; Pharmacia). The studies were carried out with Aβ-(1–40), Aβ-(1–40Q) or Aβ-(1–40A) at a concentration of 300 μM. A stock solution of apoE was made at 0.7 mg/ml in 0.1 M Tris/HCl, pH 7.4 and stored in 50 μl portions at −20 °C. For the co-incubation experiments, 1–4 μl of apoE stock solution was added to freshly resuspended samples of peptides in the same buffer and incubated at room temperature for the indicated intervals, using a final volume of 35 μl. Fluorimetry was performed as described [26,27]. The incubated samples were added to 50 mM glycine (pH 9)/2 μM Th-T (Sigma) in a final volume of 2 ml. Fluorescence was measured at excitation 435 nm and emission 485 nm in a Hitachi F-2000 fluorescent spectrophotometer. A time scan of fluorescence was performed and three values that reached a plateau after the decay (280, 290 and 300 s) were averaged after subtracting the background fluorescence of 2 μM Th-T. Fluorimetric data from two to three identical samples in separate experiments were averaged to provide the final plotted values. For co-incubation experiments, fluorescence of apoE alone preincubated at a concentration of 10 μM was determined.

Electron microscopy

Aβ peptides alone or preincubated with apoE at the concentrations mentioned above were incubated for 4 days at room temperature and placed on carbon/formvar-coated 300-mesh nickel grids (Ladd) for 1 min, blotted and stained with 2% uranyl acetate (Ladd) for 30 s. Grids were visualized in a Zeiss EM10 electron microscope at 80 kV.

Congo Red staining

Samples were placed on to gelatin-coated slides, fixed in ethanol and stained for 3–6 h with 1% Congo Red [29] dissolved in 80% ethanol saturated with NaCl, pH 11. After being washed in 80% ethanol, slides were visualized under polarized light.

Western blots and sequencing

ApoE–Aβ complexes were detected by separation in 16% Tris/tricine gels and Western-blot analysis as described [18]. The primary antibodies used were a goat polyclonal anti-apoE (Calbiochem) and a rabbit polyclonal antibody against residues 1–28 of Aβ (anti-SP-28) [30]. Immunoreactive bands were detected by enhanced chemiluminescence (Amersham). In addition, Aβ–apoE complexes detected by separation on a 16% Tris/tricine gel were transferred to a poly(vinylidene difluoride) membrane (Immobilon; Millipore Corp.), followed by excision and N-terminal sequence analysis.

RESULTS

After incubation for 24 h at 300 μM in 0.1 M Tris/HCl, pH 7.4, aggregated Aβ-(1–40) showed typical green birefringence on Congo Red staining. When these aggregates were subjected to the Th-T fluorimetric assay, a novel emission signal was observed at approximately 485 nm, as previously described [26,27]. Freshly resuspended Aβ-(1–40) in the fluorimetry buffer or preincubated in 6 M guanidinium chloride for 24 h showed no specific Th-T fluorescence at emission 485 nm. In the same way, ubiquitin or BSA incubated for 24 h at 500 μM displayed background fluorescence values (not shown). These results are consistent with reported data [26] and are highly suggestive that the Th-T fluorescent shift is related to the β-pleated secondary structure of aggregated Aβ-(1–40).

In order to correlate further Th-T fluorescence with amyloid formation, the Dutch variant Aβ-(1–40Q) was used. Similar variant peptides have been shown to be highly amyloidogenic *in vitro*, forming more stable fibrils and a faster rate than Aβ-(1–40) [13–15,31]. After incubation for 24 h, Aβ-(1–40Q) formed large sheet aggregates that showed intense green birefringence after Congo Red staining. When these two peptides were incubated in parallel at 300 μM for 24 h, Aβ-(1–40Q) showed a fluorescence yield 2-fold higher than Aβ-(1–40). Time course experiments showed higher fluorescence values throughout the entire incubation period and this difference was maintained at 24 h (Figure 1). This result suggests that Aβ-(1–40Q) not only formed amyloid at an accelerated rate but also that it reached an

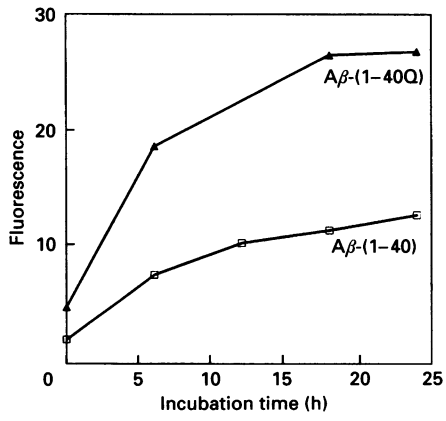

Figure 1 Th-T fluorescence of Aβ-(1–40) (□) and Aβ-(1–40Q) (▲) at different time points

Aβ-(1–40) or Aβ-(1–40Q) was incubated at a concentration of 300 μM in 0.1 M Tris/HCl, pH 7.4, over 24 h. Separate samples, in triplicate, were taken at the indicated times and subjected to fluorimetry as described in the Experimental section.

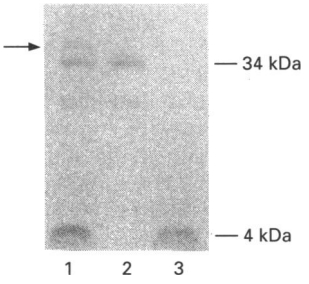

Figure 2 Coomassie-stained 16% Tris/tricine gel on which apoE and Aβ-(1–40) were run under non-reducing conditions

Lane 1, 10 μg of apoE and Aβ(1–40) were run in 2% SDS sample buffer after incubation for 8 h at 37 °C in PBS. Lane 2, apoE alone was run and is seen as a 34 kDa bond. The band at 26 kDa is a degradation product of apoE. Lane 3, Aβ-(1–40) alone is seen as a 4 kDa band. In lane 1 a supplementary band of apoE–Aβ complex is evident at approx. 40 kDa (arrow).

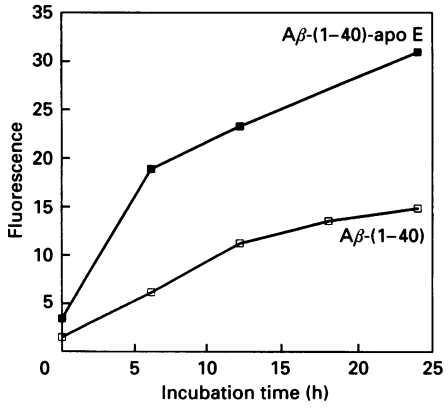

Figure 3 Th-T fluorescence of Aβ-(1–40) (□) alone and with apoE (■) at different incubation times

Aβ-(1–40) at a concentration of 300 μM was incubated in 0.1 M Tris/HCl, pH 7.4, either alone or in the presence of 3.0 μM apoE. Th-T fluorescence was determined at the times indicated as described in the Experimental section. The mean values of three separate determinations are shown.

equilibrium with a higher mass of peptide aggregated into amyloid than Aβ-(1–40).

Incubation of Aβ-(1–40) with purified human apoE for 12 h resulted in the appearance on SDS/PAGE of a new band of approximate molecular mass 40 kDa (Figure 2) consistent with an apoE–Aβ-(1–40) complex resistant to SDS treatment as previously reported [18]. Sequencing of this supplementary band revealed the N-terminal sequence of both Aβ and apoE: KVEQAVETEPEPE (apoE) and DAEFRHDSGYEVH (Aβ). In order to test the effect of this interaction on Aβ-(1–40) aggregation, the peptide was incubated at a concentration of 300 μM with 3 μM apoE for different incubation times. Aβ-(1–40) preincubated with apoE displayed fluorescence values that were between 2- and 3-fold higher than for Aβ-(1–40) alone (Figure 3). ApoE alone incubated at 3–10 μM or ubiquitin incubated with apoE showed no fluorescence signal above background, nor did Aβ incubated with BSA (results not shown). This result is consistent with a more rapid aggregation of Aβ-(1–40) in the presence of apoE and, as with the Dutch variant of Aβ, a larger amount of Aβ-(1–40) assembled into β-pleated sheet after the 24 h incubation period.

Table 1 Effect of apoE on the Th-T fluorescence of Aβ analogues containing different substitutions

Samples of each peptide at 300 μM were incubated either alone or in the presence of 2.5 μM apoE. Values represent the Th-T fluorescence and are means (±S.D.) of three separate experiments performed in triplicate.

	Th-T fluorescence			
	Alone		Plus apoE	
Peptide	6 h	24 h	6 h	24 h
Aβ-(1–40)	6.6±0.6	14.0±1.8	18.9±2.7	29.9±1.5
Aβ-(1–40Q)	21.4±3.5	29.6±2.9	27.8±1.3	33.9±2.1
Aβ-(1–40A)	0.5±0.04	0.6±0.1	7.6±0.06	10.5±0.1

In order to mimic a physiological ionic environment, pooled normal human cerebrospinal fluid was ultrafiltered through a 1 kDa membrane and the resultant ultrafiltrate, pH 7.3, was used as the incubation buffer. A similar effect of apoE on the fluorescence enhancement of Aβ-(1–40) at the same incubation times was seen as that with 0.1 M Tris/HCl, pH 7.4 (results not shown). This indicates that such interactions are possible in ionic conditions similar to those present in the extracellular fluid of the brain.

To study further the effect of apoE on amyloid formation, we analysed the changes in the Th-T fluorescence produced by incubation of apoE with Aβ analogues containing single amino acid substitutions. Incubation of apoE with Aβ-(1–40Q) for 12 h resulted in the appearance on SDS/PAGE of a new band of approximate molecular mass 40 kDa which was recognized by both anti-apoE and a polyclonal anti-Aβ antibody (anti-SP-28) (results not shown). This band was consistent with a complex formed by apoE and Aβ-(1–40Q). Fluorimetry of Aβ-(1–40Q) incubated with apoE at 300 μM and 3 μM respectively showed no significant differences after 6 or 24 h (Table 1). This result indicates that apoE had little effect on the self-aggregation of the highly amyloidogenic Dutch variant.

We further studied the effect of apoE on Th-T-fluorescence-monitored amyloid formation by using an Aβ analogue in which valine is replaced by alanine at position 18 [Aβ-(1–40A)]. This peptide shows a higher content of α-helical conformation at its N-terminus, less tendency to aggregate and requires incubation at higher concentrations (600–800 μM) to display Congo Red green birefringence after 24 h [28]. SDS/PAGE of Aβ-(1–40A) incubated with apoE for 12 h showed a 40 kDa band similar to Aβ-(1–40Q), consistent with an apoE–Aβ-(1–40A) complex. When Aβ-(1–40A) was incubated for 24 h at a concentration of 300 μM, no fluorescence above background levels was detected. Co-incubation with apoE (3 μM) resulted in a 15–17-fold increase in fluorescence after 6 and 24 h (Table 1). These results indicate that apoE produces a bigger effect on those peptides that have less β-sheet structure [Aβ-(1–40A)], consistent with the proposed idea that apoE seeds fibril growth and promotes amyloid formation [23].

To study the morphology of the amyloid fibrils produced in the presence of apoE, electron-microscopy studies were performed. Both Aβ peptides alone and those incubated with apoE formed amyloid-like fibrils. Aβ peptides alone formed fewer fibrils (Figure 4a); these were approximately 10 nm in width, unbranched and up to 3 μm in length. Aβ peptides with apoE revealed a larger number of fibrils which tended to produce a

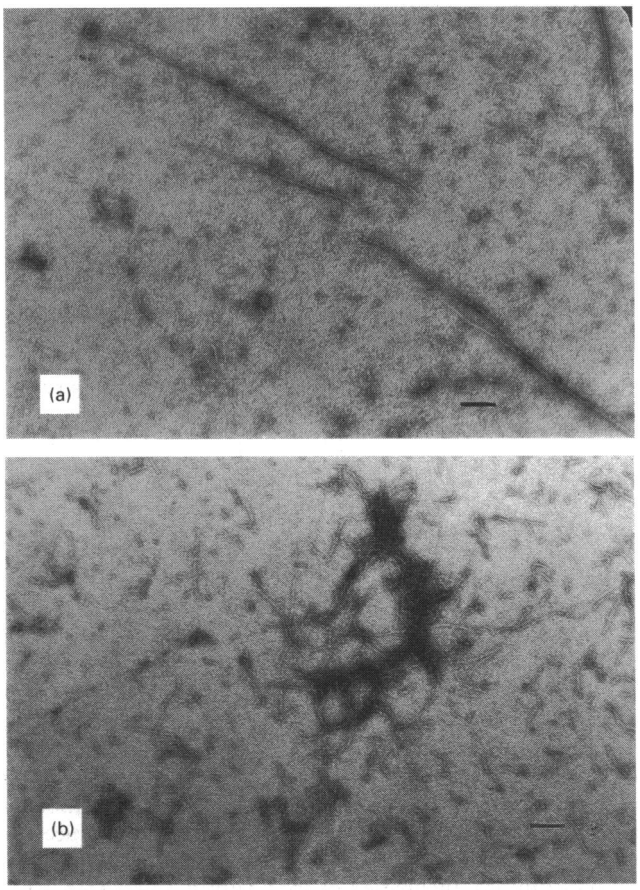

Figure 4 Electron micrographs of negatively stained Aβ-(1–40) fibrils (a) and Aβ-(1–40) with apoE (b)

The fibrils were incubated for 4 days at room temperature as described in the Experimental section. The scale bar represents 100 nm (1000 Å).

Table 2 Th-T fluorescence of Aβ-(1–40) incubated with different molar ratios of apoE isoforms

Aβ-(1–40) at 300 μM in a final volume of 15 μl was incubated for 24 h either alone or in the presence of 3 μM or 30 μM rapoE3 or rapoE4. The fluorescence value for the peptide alone was 6.8 ± 0.33. Values represent Th-T fluorescence and are means (± S.D.) of three separate experiments performed in triplicate.

apoE isoform incubated with Aβ-(1–40)	Th-T fluorescence	
	100:1	10:1
apoE3	7.06 ± 0.19	15.42 ± 1.86
apoE4	13.62 ± 1.01	39.56 ± 0.96

matrix-like meshwork of 7–10 nm fibrils of variable length (Figure 4b). No fibril formation was noted with apoE alone.

Recent data have shown a strong association between the APOE allele ε4 and both familial and sporadic Alzheimer's disease [17,18]. Furthermore, the inheritance of the APOE4 allele has been associated with an earlier age of onset and a greater Aβ immunoreactivity in an allele-dose-dependent manner [19]. We studied the comparative effect produced by recombinant isoforms of apoE on amyloid formation *in vitro*. The apoE isoforms used in this study correspond to recombinant proteins synthesized in baculovirus using the cDNA sequence of human apoE. In a molar ratio of Aβ-(1–40)/apoE of 100:1, the peptide incubated with recombinant apoE4 (rapoE4) showed the highest Th-T fluorescence value, consistent with a 2-fold increase in amyloid formation in comparison with the peptide alone or incubated with rapoE3 (Table 2). This effect was even higher when the molar ratio was reduced to 10:1 (Table 2).

DISCUSSION

The results indicate that purified human apoE can induce *in vitro* an enhancement of amyloid formation from synthetic analogues of the major form of soluble Aβ under the conditions tested. The sensitive and semiquantitative method of Th-T fluorescence permitted the detection of significant differences at early times of incubation when other methods such as Congo Red staining or electron microscopy could not distinguish such effects accurately. Moreover, the morphological findings of dense arrays of fibrillar material after co-incubation of Aβ-(1–40) with apoE compared with the loose aggregation of fibrils displayed by Aβ-(1–40) alone further supported the fluorescence findings.

Recent *in vitro* studies suggest that Aβ amyloid formation from its monomeric precursor is a nucleation-dependent polymerization process that resembles the physiological assembly of microtubules [32,33]. In this mechanism, once the nucleus is formed, polymer growth follows autocatalytic kinetics. We therefore reasoned that apoE could trigger and/or nucleate the amyloid cascade, is a similar way to the addition of preformed fibrils. Our data show that apoE obtained from the human plasma at a molar ratio 100:1 was able to induce a 2–3-fold increase in the Th-T fluorescence of Aβ-(1–40). Moreover, apoE induced a higher increase (15–17-fold) in fluorescence on a poorly amyloidogenic Aβ analogue created by substituting alanine for valine at position 18. This result, obtained at incubation times at which this peptide showed no detectable fluorescence, is consistent with a seeding effect of apoE on the ordered aggregation of Aβ-(1–40A). The small effect on the Dutch variant can be explained by the higher amyloidogenic potential resulting from glutamate to glutamine substitution at position 22 [13,14]. Hence this peptide is already self-nucleating fibril growth at an accelerated rate and no further enhancement by apoE can be seen. This observation is in agreement with the recently reported lack of association of apoE isotypes and the clinical feature of the Dutch variant of Alzheimer's disease [34].

Our findings that the enhancement of Aβ amyloid formation is higher with rapoE4 is consistent with recent reports [35,36] and provide a possible explanation for the genetic association between the ε4 allele and Alzheimer's disease. The enhancement of fibrillogenesis produced by rapoE isoforms was less than the increase induced, under the same conditions, by apoE purified from human plasma, which contains mainly apoE3. These findings suggest that rapoE isotypes could be less active as a result of partial denaturation during the purification process or that human plasma apoE may contain post-translational modifications that are absent from the recombinant forms.

In biological fluids the molar ratio of Aβ/apoE is opposite to that used in these experiments. Our yield of apoE C-terminal end extracted from amyloid deposits from two patients with systemic amyloidosis is close to 100:1 of amyloid protein to apoE (F. Prelli & E. Castaño, unpublished work), and therefore this ratio was used to mimic the conditions that may occur locally in the diseased brain in which high concentrations of Aβ are present at early stages of the disease as preamyloid lesions. The interaction between apoE and Aβ is likely to depend on both the

concentration and conformational state of these proteins; hence, under different experimental conditions, other results may be obtained. For example, a recent report shows that apoE may inhibit amyloid formation by Aβ under different experimental conditions [37].

ApoE is a well-characterized two-domain protein modelled by its two main products after thrombin digestion. The 22 kDa N-terminal domain (residues 1–191) is a stable globular structure containing the sequence that mediates binding of low-density lipoprotein receptor [38]. In contrast, the 10 kDa C-terminal thrombolytic domain is less stable, binds to lipoproteins and mediates apoE tetramerization in aqueous solution. The region of apoE between residues 244 and 272 within the lipid-binding domain of the C-terminus is capable of binding to synthetic Aβ via a strong hydrophobic interaction resistant to SDS treatment [39]. We have extended study of this *in vitro* binding of apoE to the Dutch variants of Aβ and Aβ-(1–40A) analogue. Recent kinetic studies on synthetic Aβ aggregation by light scattering suggest that Aβ monomers or dimers can self-assemble rapidly into octamers that in turn grow end to end into amyloid fibrils at a lower rate [32]. These findings together with the results in our study are consistent with a model in which apoE, possibly through its C-terminus, promotes early side-chain interactions between Aβ monomers or oligomers which lead to accelerated assembly of β-pleated sheet. Alternatively, apoE may facilitate end-to-end growth of the nascent amyloid fibrils.

Toxicity of Aβ towards neurons in culture has been used as a model to propose a pathogenic role for this peptide in Alzheimer's disease [40], where it has been shown that the aggregation of Aβ influences the toxic effect [41]. Our *in vitro* results suggest that apoE may constitute an important cofactor in Aβ fibrillogenesis and therefore may influence its toxicity. In addition, it has been suggested that apoE4 inhibits neurite outgrowth, compared with apoE3 [42]. However, another report has shown that, in cultured cells, apoE attenuates the neurotoxicity of Aβ [43]. These apparently contradictory results could be due to the different experimental conditions used.

One of the current hypotheses about Alzheimer's disease states that certain apoE genetic isoforms are involved in formation of neurofibrillary tangles [21]. In this regard, it has been proposed that the apoE3 isoform may have a protective role by binding to tau protein, preventing this protein from abnormal phosphorylation and self-aggregation into PHF [21]. Moreover, apoE has been localized in close association with neurofibrillary tangles [23,24,44]. If an interaction like the one reported here between apoE and Aβ occurs between apoE and abnormally accumulated tau protein within neurons, apoE may represent a shared promoter in the process of fibrillogenesis of PHF and Aβ.

ApoE is ubiquitous and is associated with all kinds of cerebral and systemic amyloids [23]. Therefore the interaction that we show here using Aβ analogues as a model system may be similar to other amyloid proteins regardless of their primary structure. It is possible, however, that a certain specificity exists in the interaction between apoE4 and Aβ. In the same way, further studies are needed to clarify whether other exchangeable apolipoproteins containing similar amphipathic helices (e.g. apolipoproteins AI and Cs) are also able to nucleate fibril growth of amyloidogenic peptides, reflecting a more general amyloid–apolipoprotein interaction. In this regard, it is noteworthy that variants of apolipoprotein AI can form amyloid in certain hereditary forms of human amyloidosis [45]. In addition, intact human apolipoprotein AI can spontaneously form amyloid fibrils *in vitro* (T. Wisniewski, unpublished work). The specificity of the abnormal amyloid response will be finally determined by a complex process involving demonstration of high local concentrations of amyloid precursors and other amyloid-associated proteins such as apoE, α_1-antichymotrypsin, amyloid P component, protease inhibitors or proteoglycans in conjunction with as yet unknown tissue-specific factors. It is our hypothesis that a number of different factors can nucleate Aβ fibril growth, including longer Aβ species [Aβ-(1–42/44)], apoE (apoE4 in particular), other apolipoproteins such as AI, or other chaperones such as α_1-antichymotrypsin. The importance of each of these factors will vary among individual cases of Alzheimer's disease.

In summary, the present study examined the *in vitro* effect of low concentrations of apoE on highly amyloidogenic concentrations of synthetic peptides analogous to the major soluble form of Aβ. Our results indicate that apoE, in particular apoE4, may act as a 'pathological chaperone' [23] by promoting the β-pleated-sheet assembly of soluble Aβ. Dissecting the complex interactions between this peptide and its associated proteins will shed light on the mechanisms that result in brain amyloid deposition and may open up new strategies for the development of potential therapeutic intervention.

Note added in proof (received 9 January 1995)

Since this manuscript went for proofing-out, recent studies in our laboratory have shown that the C-terminal end of apoE (residues 216–299) is found *in vivo* as a component of neuritic plaques and can form, by itself, amyloid-like fibrils *in vitro*.

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