# SDS-Stable Complex Formation between Native Apolipoprotein E3 and $\beta$-Amyloid Peptides[†]

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ABSTRACT: Extracellular senile plaques composed predominantly of fibrillar amyloid-$\beta$ (A$\beta$) are a major neuropathological feature of Alzheimer's disease (AD). Genetic evidence and in vivo studies suggest that apolipoprotein E (apoE) may contribute to amyloid clearance and/or deposition. In vitro studies demonstrate that native apoE2 and E3 form an SDS-stable complex with A$\beta$(1−40), while apoE4 forms little such complex. Our current work extends these observations by presenting evidence that apoE3 also binds to A$\beta$(1−42) and with less avidity to modified species of the peptide found in senile plaque cores. These modified peptides include a form that originates at residue 3-Glu as pyroglutamyl and another with isomerization at the 1-Asp and 7-Asp positions. In addition, we used binding reactions between apoE3 and various A$\beta$ fragments, as well as binding reactions with apoE3 and A$\beta$(1−40) plus A$\beta$ fragments as competitors, to identify the domain(s) of A$\beta$ involved in the formation of an SDS-stable complex with apoE3. Residues 13−28 of A$\beta$ appear to be necessary, while complex formation is further enhanced by the presence of residues at the C-terminus of the peptide. These results contribute to our understanding of the biochemical basis for the SDS-stable apoE3/A$\beta$ complex and support the hypothesis that A$\beta$ can be transported in vivo complexed with apoE. This complex may then be cleared from the interstitial space by apoE receptors in the brain or become part of an extracellular amyloid deposit.

Extracellular senile plaques composed predominantly of fibrillar amyloid-$\beta$ (A$\beta$) are a major neuropathological feature of Alzheimer's disease (AD).[1] Analysis of these deposits reveals that apolipoprotein E (apoE) is a major non-amyloid component, associated with amyloid in stable complexes (*1*−*3*). A$\beta$, a 39- to 43-residue peptide formed by proteolytic cleavage of amyloid precursor protein (APP) is also found in a soluble, non-fibrillar form in human brain, cerebral-spinal fluid (CSF), plasma, and urine (*4*−*7*). This soluble A$\beta$ has been shown to immunoprecipitate with apoE (*8*−*10*). ApoE, a component of several classes of lipoproteins, acts as a ligand for lipoprotein receptors, thus regulating lipid transport and clearance. In humans, apoE is a 299-amino acid (∼35 kDa) secretory protein that has three major isoforms, E2 ($Cys^{112}$, $Cys^{158}$), E3 ($Cys^{112}$, $Arg^{158}$), and E4 ($Arg^{112}$, $Arg^{158}$), products of three alleles ($\epsilon$2, $\epsilon$3, and $\epsilon$4) at a single gene locus. The $\epsilon$4 allele is present with increased frequency in patients with sporadic and late-onset familial AD (*11*−*13*), making apoE4 a risk factor for the disease.

The exact mechanism by which apoE and A$\beta$ contribute to the pathogenesis of AD is not yet entirely understood. However, a physical association between apoE and A$\beta$ may serve as either a means of clearing the peptide via the recognition of apoE/A$\beta$ complexes by apoE receptors (*14*, *15*) or apoE may act as a pathological chaperone, facilitating the deposition of amyloid (*16*, *17*). In vitro, apoE interacts with A$\beta$ to form a stable complex (*8*, *11*, *15*, *18*−*20*), alters the aggregation of the A$\beta$ peptide (*21*−*23*), and affects A$\beta$-induced neurotoxicity (*24*−*26*). On the basis of these published accounts, the effect, if any, of apoE isoform on the interaction between apoE and A$\beta$ is unclear. The difference in apoE/A$\beta$ interactions can be attributed in part to the source of apoE. For example, in biochemical assays, native apoE2 and E3 (associated with lipid particles) form an SDS-stable complex with A$\beta$(1−40) that is more abundant than apoE4/A$\beta$ complex (*15*, *18*, *20*, *27*, *28*). However, purified apoE3 and E4 exhibit a comparable and lower affinity for the peptide (*11*, *18*). These results measuring SDS-stable complex formation by gel-shift assay have recently been confirmed using a solid-phase binding assay (*29*).

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[1] Abbreviations: A$\beta$, amyloid-$\beta$; AD, Alzheimer's disease; apoE, apolipoprotein E; APP, amyloid precursor protein; CSF, cerebral-spinal fluid; PAGE, polyacrylamide gel electrophoresis; BCA, bicinchoninic acid; ECL, enhanced chemiluminescence; 1,7-isoAsp, A$\beta$(1−42) with isomerized aspartic acid residues at positions 1 and 7; 3pE, A$\beta$(1−42) starting at residue 3-glu in the form of pyroglutamyl; 13, 14-Gln, A$\beta$-(1−42) with the histidines at positions 13 and 14 changed to glutamine; HSPG, heparan sulfate proteoglycans.

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Our working hypothesis is that apoE interacts in an isoform-specific manner with A$\beta$ to form an SDS-stable complex that results in the differential clearance and/or deposition of the peptide. The relevance of SDS-stable apoE/A$\beta$ complexes is further supported by in vivo data from Russo and co-workers who demonstrated that nearly all A$\beta$ in plaque-free non-AD brain samples is bound in SDS-stable complexes with apoE, while AD brains contain significantly greater amounts of free A$\beta$ and less stable apoE/A$\beta$ complexes (*30*). They also report that A$\beta$ in apoE/A$\beta$ complexes is more susceptible to proteases. Thus, AD brains may fail to degrade A$\beta$ via an apoE-mediated process, leading to neurotoxic levels of the peptide or a toxic aggregate.

Prior in vitro studies on SDS-stable apoE/A$\beta$ complex formation have focused primarily on interactions between apoE and A$\beta$(1−40). However, the most prevalent form of the peptide found in senile plaques is A$\beta$(1−42), which is modified after cleavage from APP. Of the total A$\beta$ found in amyloid plaque cores, ∼51% starts at residue 3-Glu in the form of pyroglutamyl (*31*), and ∼60% of the remaining A$\beta$ starts at position 1-Asp and are substantially isomerized (*32*, *33*). In addition, ∼75% of the total A$\beta$ in amyloid plaques contains isomerized Asp at position 7 (*31*). We present data that apoE3 binds to A$\beta$(1−42) and less avidly to the modified forms of this peptide found in senile plaques.

Attempts to identify the region(s) of A$\beta$ involved in complex formation with apoE have been limited, and results using several different assay systems suggest that various regions of A$\beta$ are involved (*11*, *34*, *35*). Here we extend our previous observations on SDS-stable apoE/A$\beta$ complex formation by identifying the domain(s) of A$\beta$ involved in complex formation with apoE3.

## MATERIALS AND METHODS

*Expression of Human apoE3 in Cultured Cells.* Serum-free conditioned media from HEK-293 cells stably transfected with human cDNA encoding apoE3 was prepared as described (*15*). Conditioned medium containing apoE3 was concentrated (Centriprep, Amicon, Inc.) ∼50-fold and fractionated by gel chromatography, and the resulting fractions containing apoE particles were pooled. The amount of apoE3 was quantified by SDS−polyacrylamide gel electrophoresis (SDS−PAGE), protein staining, and densitometry (ImageQuant, Molecular Dynamics, Inc.) of serial dilutions of apoE3-containing samples using a purified apoE3 standard. ApoE3 was used for all the data presented herein as previous results indicated that apoE3 formed an SDS-stable complex with A$\beta$(1−40) that was easily detected by SDS−PAGE and Western analysis (*15*, *18*).

*A$\beta$ Peptides.* A$\beta$ peptides were purchased from either California Peptide Research Inc. (Napa, CA) or Quality Controlled Biochemicals, Inc. (Hopkinton, MA) and purified by FPLC or by reverse phase HPLC (*31*, *32*). The composition of the peptides was characterized by mass spectrometry, automatic amino acid analysis, and, when required, HPLC peptide mapping of tryptic fragments.

*ApoE3/A$\beta$ Complex Formation and Detection.* For binding reactions, synthetic A$\beta$ peptides were resuspended to 5 mM in 100% $Me_2SO$. The concentrations of the resuspended peptides were verified using a bicinchoninic acid (BCA) assay (Sigma), and reactions were loaded based on these calculations to ensure equimolar use of peptides. ApoE3 (25 $\mu$g/mL, ∼700 nM) was incubated for 2 h at room temperature with 250 $\mu$M A$\beta$ at pH 7.4 as described previously (*15*). Control reactions without A$\beta$ contained 5% $Me_2SO$. Reactions were stopped by addition of 2× non-reducing Laemmli buffer (*36*) (4% SDS, no $\beta$-mercaptoethanol) and frozen at −20 °C. Samples were boiled 5 min, electrophoresed on 10−20% SDS−PAGE gels, transferred to Immobilon-P membranes (Millipore), and probed with antibodies to apoE (1:5000 dilution) or A$\beta$. Monoclonal antibody 4G8 (to amino acids 17−24 of A$\beta$) was purchased from Senetek PLC (Maryland Heights, MO) and used at 1:5000 dilution. Monoclonal antibody 1702.1 raised against the terminal amino acid of A$\beta$(1−40) was generously provided by Barbara Cordell (Scios, Inc) and used at 1:500 dilution. ApoE antisera were obtained by immunizing rabbits with apoE purified from human serum. For binding reactions with competitor peptides, the apoE3 was pre-incubated for 2 h with the various competition peptides (250 $\mu$M) after which 250 $\mu$M A$\beta$(1−40) was added for an additional 2 h incubation. Proteins on Western blots were visualized by enhanced chemiluminescence (ECL; Amersham Corp.) and quantified by densitometry (ImageQuant, Molecular Dynamics, Inc.).

*Correcting for Differences in Antibody Affinity.* The concentrations of all A$\beta$ peptides and peptide fragments were determined by the BCA protein assay, and these values were used to ensure equal loading in the various reactions. However, 4G8 and 1702.1 did not recognize all of the peptides used in Figure 2 with equal affinity. Therefore, the relative affinity of 4G8 and particularly 1702.1 for the monomeric forms of the wild type or peptide fragments was determined by loading peptide alone based on BCA protein determinations and detection by Western analysis as described above (data not shown). This factor was then used as a correction factor for determining the relative amount of SDS-stable apoE3/A$\beta$ complex for comparison between the peptide fragments. This correction assumes that the affinity of the antibody is the same for free A$\beta$ and A$\beta$ bound to apoE. This correction is reflected in the proportions reported in the results section for Figure 2.

## RESULTS

*SDS-Stable Complex Formation between ApoE and Modified A$\beta$(1−42).* To characterize the interactions between apoE3 and A$\beta$(1−42), we incubated apoE3 with the wild-type peptide and with modified A$\beta$(1−42) peptides found in vivo, A$\beta$(1−42) with isomerized aspartic acid residues at positions 1 and 7 (1,7-isoAsp), and A$\beta$(1−42) starting at residue 3-glu in the form of pyroglutamyl (3pE) (Figure 1). For the gel shown in Figure 1, the modified peptides were loaded at 3- and 1.5-fold, respectively, the concentration of the wild-type A$\beta$(1−42) as determined by BCA assay. This was done in an effort to detect complex from the wild type and the modified peptides on the same exposure. The A$\beta$-(1−42) monomer and dimer appear at ∼4 and ∼8 kDa (Figure 1, panel B, lane 1), as do the monomers and dimers for the modified peptides that also contain stable aggregates of ∼16 and ∼200 kDa (Figure 1, panel B, lanes 4−6). 1,7-isoAsp also contain an apparent degradation fragment at ∼2.5−3kDa (Figure 1, panel B, lane 5). Under the nonreducing SDS−PAGE conditions utilized, apoE3 monomer

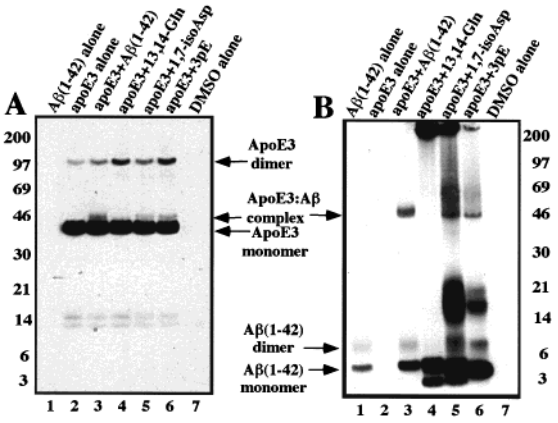


FIGURE 1: SDS-stable complex formation between apoE and modified A$\beta$(1−42). Western blots of 250 $\mu$M A$\beta$(1−42) (lane 1); 25 $\mu$g/mL apoE (∼700 nM, lane 2); 25 $\mu$g/mL apoE incubated for 2 h at room temperature with either 250 $\mu$M A$\beta$(1−42) (lane 3), A$\beta$(13,14-Gln) (lane 4), A$\beta$(1,7-isoAsp) (lane 5), or A$\beta$(3pE) (lane 6); and a control lane containing 5% $Me_2SO$ (lane 7). Molecular masses are given in kDa. Samples were run in non-reducing Laemmli buffer on 10−20% SDS−PAGE gels, transferred to Immobilon-P membrane, and probed with apoE antisera (A) or 4G8 antibody (to A$\beta$ residues 17−24) (B).

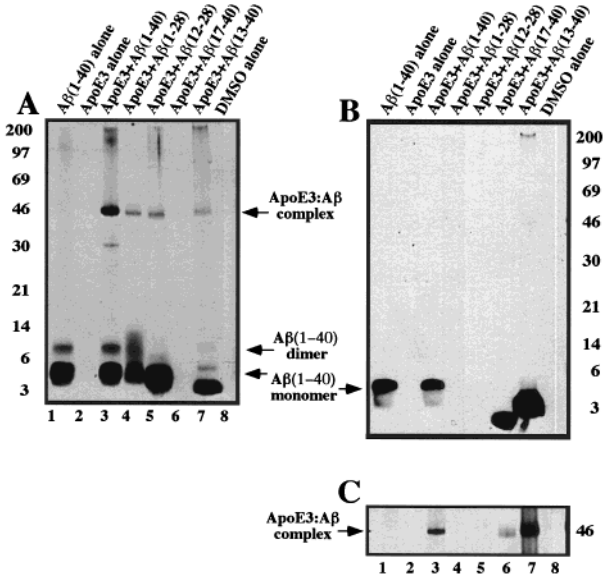


FIGURE 2: SDS-stable complex formation between apoE3 and A$\beta$ peptide fragments. Western blots of 250 $\mu$M A$\beta$(1−40) (lane 1); 25 $\mu$g/mL apoE (∼700 nM, lane 2); 25 $\mu$g/mL apoE incubated with either 250 $\mu$M A$\beta$(1−40) (lane 3), A$\beta$(1−28) (lane 4), A$\beta$(12−28) (lane 5), A$\beta$(17−40) (lane 6), and A$\beta$(13−40) (lane 7). Lane 8 (control) contained 5% $Me_2SO$. Protocol as described in legend to Figure 1, 4G8 antibody (A) or 1702.1 antibody [to residue 40 of A$\beta$(1−40)] (B). Panel C is a longer exposure of the ∼45-kDa region of the gel shown in panel B. Molecular masses are given in kilodaltons.

appears at ∼35 kDa, while apoE3 dimer is ∼90 kDa (Figure 1, panel A, lane 2).

When A$\beta$(1−42) was incubated with apoE3, both apoE antiserum and antibody 4G8 detected an SDS-stable immunoreactive complex that migrated at ∼45 kDa or slightly above ∼35 kDa apoE3 monomers (Figure 1, panels A and B, lane 3). This is the pattern previously observed for SDS-stable complex formation between apoE3 and A$\beta$(1−40) under comparable conditions (*15*, *20*, *28*). Similarly sized apoE3/A$\beta$ complexes were detected for 1,7-isoAsp and 3pE, though at levels reduced to ∼20% of unmodified A$\beta$(1−42), as determined by calibrated densitometry of data such as those presented in Figure 1, panel B. As a control for the amount of A$\beta$ in the modified peptides that was present as aggregates and thus possibly unavailable for binding to apoE, the fraction of A$\beta$ bound to apoE was calculated as a ratio to A$\beta$ monomer. Thus, apoE can form an SDS-stable complex with A$\beta$(1−42) but has less avidity for the modified forms of the peptide most abundant in amyloid plaque cores. In addition, the ability of 3pE and 1,7-isoAsp to form SDS-stable complexes with apoE3 suggests that residues 1−3 and residues 1 and 7 may not be absolutely critical for complex formation between A$\beta$ and apoE3.

A$\beta$(1−42) with the histidines at positions 13 and 14 changed to glutamine (13,14-Gln) was used to further dissect the regions of A$\beta$ important to complex formation with apoE3. This modified peptide was originally created after an earlier study found that positively charged histidines at residues 13 and 14 were within the putative heparan sulfate proteoglycans (HSPG) binding domain and important in microglia activation (*37*). For the gel shown in Figure 1, 13,14-Gln was loaded at 4-fold the concentration of the wild-type peptide. We observed a >99% decline in complex formation between control A$\beta$(1−42) and 13,14-Gln (Figure 1, panel B, lane 4; longer exposures not shown were used for detection of the apoE/13,14-Gln complex). These results suggest that the HSPG binding domain of A$\beta$ is critical to complex formation with apoE. The interaction between apoE and A$\beta$ may be charge-dependent, given that the replacement of these two histidines with glutamines results in the loss of two positive charges. It remains to be determined whether the difference in charge affects binding directly or indirectly via a change in domain interactions that affect the conformation of A$\beta$ prior to incubation with apoE3.

*SDS-Stable Complex Formation between ApoE3 and A$\beta$ Peptide Fragments.* To further identify the regions of A$\beta$ that are involved in SDS-stable complex formation with apoE3, we measured the relative amounts of complex formed between apoE3 and peptide fragments that contained a domain recognized by either A$\beta$ monoclonal antibody 4G8 (residues 17−24) or monoclonal antibody 1702.1 that identifies A$\beta$ ending at residue 40.

ApoE3 was incubated with A$\beta$ peptide fragments 1−28, 12−28, 13−40, 17−40, and 1−40, and the relative amount of SDS-stable complex that formed was compared. A$\beta$(1−40) is primarily a 4 kDa monomer with ∼20% as a ∼8 kDa dimer under the conditions of these experiments. As expected on the basis of peptide length, A$\beta$(12−28), (13−40), and (17−40) monomers and dimers migrated more rapidly than A$\beta$(1−40) monomer and dimer and appear at ∼2−3 and ∼4−6 kDa, respectively (Figure 2, panels A and B, lanes 5−7). However, A$\beta$(1−28) monomer appears at ∼4 kDa, with no clear dimer band but rather a diffuse 4G8-immunoreactive region above the monomer (Figure 2, panel A, lane 4).

Incubation of apoE3 with these peptide fragments led to the formation of SDS-stable immunoreactive complexes at ∼45 kDa. As detected by both apoE antisera (data not shown) and 4G8, the A$\beta$(1−40) control formed more apoE3/A$\beta$ complex than any of the fragments. Complex formation with A$\beta$ peptides (1−28), (12−28), and (13−40) was reduced to ∼30% of A$\beta$(1−40) levels, as determined by calibrated

densitometry of data such as those presented in Figure 2, panel A.

Because 4G8 consistently detected only minimal amounts of A$\beta$(17−40) monomer alone or complexed to apoE3, Figure 2, panel A, was not used to measure the relative complex formation of A$\beta$(17−40). Instead, antibody 1702.1, specific for residue 40 of A$\beta$(1−40), was used to measure the amount of A$\beta$(17−40) and apoE3/A$\beta$(17−40) complex. Western blot analysis with this antibody revealed monomers for A$\beta$(1−40), (17−40), and (13−40) (Figure 2, panel B). With a longer exposure, complexes for apoE3/A$\beta$(1−40) and apoE3/A$\beta$(13−40) were readily visible, while apoE3/A$\beta$(17−40) showed only a faint amount of complex (Figure 2, panel C), consistent with minimal detection of apoE3/A$\beta$(17−40) complex using apoE antisera (data not shown). Figure 2, panel C, shows more apoE3/A$\beta$(13−40) than apoE3/A$\beta$(1−40) complex, whereas Figure 2, panel A, shows the reverse. This apparent discrepancy is likely the result of a generally higher affinity of 1702.1 (versus 4G8) for A$\beta$(13−40) in both monomeric and apoE-complexed forms as equal molar amounts of the peptides were loaded. As discussed in the methods section, the affinity of 1702.1 for peptide monomer was used as a correction factor for the amount of complex formed.

A$\beta$(1−28), (12−28), and (13−40) all form an SDS-stable complex with apoE3, though not in as great an abundance as full-length A$\beta$(1−40), suggesting that residues 1−12 are not critical for complex formation with apoE. As compared to peptide fragments containing residues 13−28, A$\beta$(17−40) formed much less complex. Thus, from these direct binding experiments, residues 13−16 appear to be important for complex formation with apoE.

*Inhibition of SDS-Stable ApoE3/A$\beta$(1−40) Complex Formation by A$\beta$ Peptide Fragments.* For peptide fragments that are not recognized by either of the A$\beta$ antibodies, we determined the relative capacity of these peptide fragments to inhibit complex formation between A$\beta$(1−40) and apoE3 as a means of further exploring the A$\beta$ domains involved in SDS-stable complex formation with apoE. ApoE3 was pre-incubated for 2 h with A$\beta$ peptide fragments prior to an additional 2 h incubation with A$\beta$(1−40). This protocol allowed peptide fragments the opportunity to bind to apoE3 and prevent subsequent A$\beta$(1−40) binding. As shown in Figure 3, panel A, the degree of inhibition was determined using antibody 1702.1 to detect the ∼45 kDa apoE3/A$\beta$-(1−40) complex but not complexes between apoE3 and the various peptides fragments, with the exception of apoE3/A$\beta$(17−40) complex.

Only pre-incubation with A$\beta$(12−42) peptide (lane 7) completely prevented formation of apoE3/A$\beta$ (1−40) complex. Of the remaining peptides, A$\beta$(1−28) and A$\beta$(13−20) reduced complex formation to 10 and 20%, respectively, of control levels, while A$\beta$(1−5), (1−12), and (1−16) had little or no effect.

The effectiveness of A$\beta$(17−40) as a competitive inhibitor is unclear. The results in Figure 3, panel A, utilize antibody 1702.1, an antibody that can detect both A$\beta$(1−40) and A$\beta$(17−40). In the presence of A$\beta$(17−40), there appears to be no complex with A$\beta$(1−40) at ∼45 kDa, suggesting that 17−40 completely inhibits 1−40 complex formation, and a small amount of complex with 17−40 at ∼42 kDa, further suggesting that the 17−40 peptide is an effective

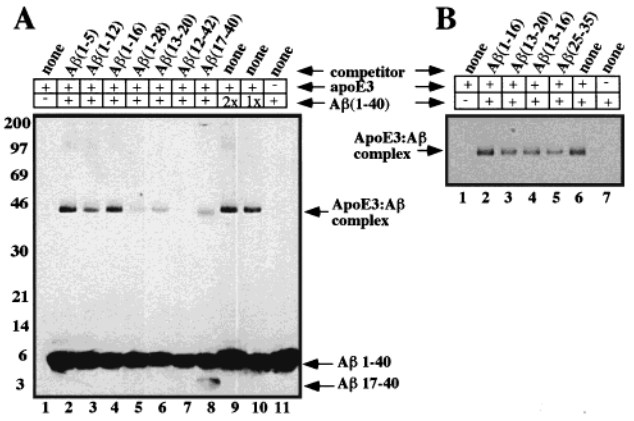


FIGURE 3: Inhibition of SDS-stable apoE3/A$\beta$(1−40) complex formation by A$\beta$ peptide fragments. ApoE was pre-incubated with A$\beta$ competitor peptides for 2 h at room temperature, followed by the standard protocol described for Figure 1 and probed with antibody 1702.1 (A) or 4G8 (B and C). Molecular masses are given in kilodaltons. (A) Western blot of 25 $\mu$g/mL apoE (∼700 nM, lane 1); 25 $\mu$g/mL apoE pre-incubated with 250 $\mu$M of A$\beta$ fragments (lanes 2−8) or A$\beta$(1−40) (lane 9); 25 $\mu$g/mL apoE incubated with 250 $\mu$M A$\beta$(1−40) for 2 h without preincubation (lane 10); and 250 $\mu$M A$\beta$(1−40) alone (lane 11). (B) Western blot of 25 $\mu$g/mL apoE (∼700 nM, lane 1); 25 $\mu$g/mL apoE pre-incubated with 250 $\mu$M A$\beta$ fragments (lane 2−5), 250 $\mu$M A$\beta$(1−40); and 250 $\mu$M A$\beta$(1−40) alone (lane 7).

competitor even though it produces only a small amount of detectable complex with apoE3 itself (Figure 3, panel A, lane 8). The small amount of apoE3/A$\beta$(17−40) complex as detected by 1702.1 is consistent with the results from the direct binding reactions (Figure 2, panel C, lane 6). Thus, the results for A$\beta$(17−40) appear paradoxical. This fragment forms little detectable complex with apoE3, though it is an effective competitive inhibitor of apoE3/A$\beta$(1−40) complex formation.

We were able to confirm and extend these results using competition assays with peptide fragments that do not contain residues 17−24, the recognition sequence for 4G8. As shown in Figure 3, panel B, peptide fragment 1−16 does not affect the formation of apoE3/A$\beta$(1−40) complex (lane 2 versus 6) but fragments 13−20, 13−16, and 25−35 exhibit comparable, modest inhibition, reducing complex formation to ∼60% of control levels (lanes 3−5 versus lane 6). The inhibition with 13−16 appears inconsistent with the lack of inhibition with 1−16 and may be the result of a conformational folding in the N-terminus that hinders the reactive area of 13−16.

In summary, the results presented in Figure 3 confirm the observations illustrated in Figure 2 that fragments containing residues 13−16 are effective competitors for A$\beta$(1−40). The peptide fragment A$\beta$(12−42) was a better competitor than 1−28 and was the only peptide fragment able to completely inhibit formation of apoE/A$\beta$(1−40) complexes. Therefore, it seems plausible that residues in the C-terminus of A$\beta$ may further facilitate complex formation with apoE3.

## DISCUSSION

The in vitro gel shift assay used to generate the data presented here has previously revealed an isoform difference in the formation of an SDS-stable complex between A$\beta$(1−40) and native apoE2 and E3 versus apoE4 (*15*, *18*, *27*). In the current results, we present evidence for the first

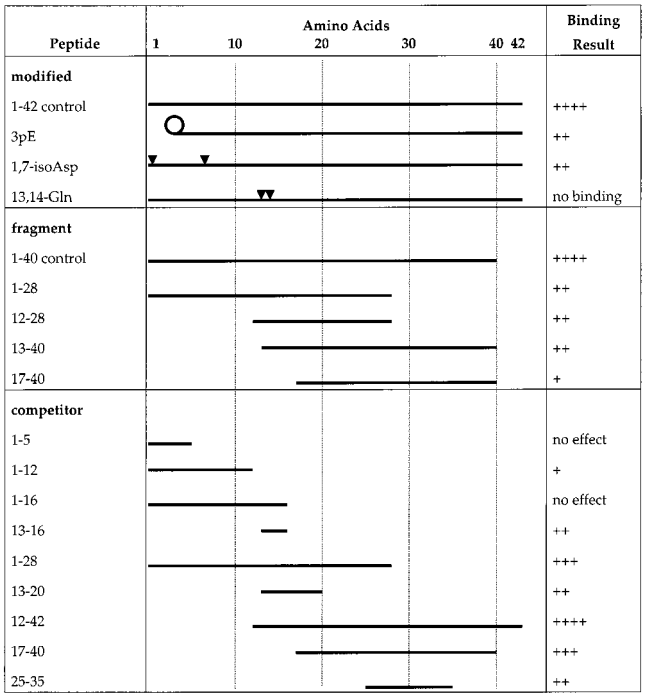


FIGURE 4: Summary figure. This figure schematically presents the results from each of the binding reactions with the various peptides. For the modified and fragment peptide reaction results, ++++ indicates maximal and + indicates minimal binding. For the competition reaction results, ++++ indicates greatest and + indicates least inhibition of apoE3/A$\beta$ (1−40) complex formation.

time that native apoE3 readily forms an SDS-stable complex with A$\beta$(1−42). In addition, apoE3 forms a complex with 3pE and 1,7-isoAsp, two modified species of A$\beta$(1−42) found in senile plaques, albeit at lower levels. These modified peptides constitute a large proportion of the total extracellular A$\beta$(1−42) in amyloid plaque cores. The amount of resident A$\beta$ in these locations is the result of their rate of production from APP and their rate of removal by cellular uptake or proteolysis. We have previously postulated that apoE plays a role in the cellular clearance of A$\beta$ via cell surface apoE receptors (*14*, *15*). The fact that apoE forms complexes less efficiently with the modified peptides than with wild-type A$\beta$(1−42) might account for their lower rate of clearance and hence accumulation in plaques.

In addition, we have used both direct binding and competition assays to begin to address which linear domains of A$\beta$(1−40) are involved in complex formation with apoE3 (summarized in Figure 4). There appears to be no specific requirement for the first seven residues of A$\beta$ as modified peptides 3pE and 1,7-isoAsp have a significant although limited capacity to form SDS-stable complexes and 1−5 has no effect in competition binding reactions with 1−40. The N-terminal edge of the active A$\beta$ peptide appears to lie between residues 13−16. The 13,14-Gln mutation completely inhibits complex formation, and A$\beta$ peptides 13−16 and 13−20 are moderately good competitive inhibitors, but A$\beta$(1−16) does not appear to compete. Although we did not determine the secondary structure of the peptides used in the present study, this ambiguity could be due to conformational differences among these peptides. For example, Golabek and co-workers have shown that apoE preferentially binds to A$\beta$ peptides with a $\beta$-sheet conformation (*38*).

Peptides extending through residue 28 are the most efficient competitors and also exhibit moderate direct binding to apoE3. These results appear to support previous reports that A$\beta$(1−28) is sufficient for complex formation with apoE using either an SDS−PAGE gel shift assay comparable to that used here (*11*) or an APP affinity column competed with A$\beta$ peptide fragments (*34*). Although 1−28 is an effective competitive inhibitor, our observations also indicate that the hydrophobic region in the C-terminus of A$\beta$ further facilitates complex formation with apoE. As peptide fragments containing residues 29−39 are highly insoluble in aqueous solutions, we have used several peptide fragments that include this domain, as well as fragment 25−35 that has additional N-terminal hydrophilic residues to facilitate solubility. A$\beta$(12−42) and A$\beta$(17−40) were among the best competitive inhibitors, and fragment 25−35 was a moderately effective competitive inhibitor. This finding is consistent with previous lipid-fusion experiments that suggested the participation of the C-terminal region of A$\beta$ in the binding to apoE (*35*). While our hypothesis is that residues in the N- and C-terminus of A$\beta$ influence complex formation by modifying the conformation of the peptide, thus affecting the exposure of the apoE binding site located in residues 13−28 of A$\beta$, it is also possible that there is not simply a single binding site. The peptide may have multiple domains that interact with apoE. For example, residues in the C-terminus of A$\beta$ may contain an apoE binding site with a lower affinity than the binding site located in residues 13−28.

Several domains of A$\beta$ have been reported to be involved in particular neurobiological activities, including residues 13−16 and the C-terminal domain as represented by A$\beta$(25−35). In the data presented here, both of these A$\beta$ regions appear to be involved in SDS-stable complex formation with apoE3. Previous observations suggest that residues 13−16 of A$\beta$ are necessary for the binding of the peptide to microglia heparan sulfate proteoglycans (HSPG), resulting in activation of these cells (*37*). Because the apoE-binding domain of A$\beta$ also appears to involve residues 13−16, apoE may function as a competitive inhibitor of A$\beta$-HSPG binding, thereby attenuating the activity of the peptide. ApoE itself has two heparan binding domains, suggesting an additional mechanism by which apoE may compete with A$\beta$ for HSPG binding. In addition, HSPG binding facilitates uptake of apoE by its receptors and may directly mediate the uptake of apoE (*39*), further evidence for an interaction between apoE, A$\beta$ and HSPG at the surface of cells that could influence the activity of the peptide, apoE and/or an apoE/A$\beta$ complex.

Both full-length A$\beta$ and A$\beta$(25−35) induce neurotoxicity, and we have shown that this toxicity is prevented by apoE3 (*25*). Our hypothesis is that apoE may form a complex with A$\beta$ in the interstitial space that can then be cleared by apoE receptors (*14*, *15*), reducing the amount of biologically active peptide available to interact with neural cells. As native apoE2 and E3, but not E4, form an SDS-stable complex with A$\beta$ (*15*, *18*, *27*), this theory provides a cellular explanation for the observed genetic correlation between $\epsilon$4 and AD. The results presented here that the C-terminus of A$\beta$ is involved in complex formation with apoE3 are consistent with this hypothesis. Thus, preferential formation of an SDS-stable complex between apoE3 and A$\beta$ may provide a mechanism for attenuating the biological activity of A$\beta$, including

neurotoxicity and the glial-mediated immunopathology associated with AD.

In summary, our results suggest that residues 13 through 28 of A$\beta$ interact with the C-terminus of the peptide to facilitate the formation of an SDS-stable complex with apoE. It is intriguing to speculate that this interaction may further affect the interaction of A$\beta$ and apoE with HSPGs and the cell surface, potentially modifying the activity of both A$\beta$ and apoE. However, the interaction between the various functional domains of apoE and A$\beta$ is undoubtedly complex, dependent upon a number of conditions that affect the conformation of the two proteins. The precise role of the specific A$\beta$ domains in both the functional activity of the peptide, as well as its interactions with apoE, must be confirmed by further in vitro and in vivo studies.

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