155

Apolipoprotein E inhibits liposome fusion induced by the Alzheimer's β-amyloid peptide

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Abstract

C-Terminal fragments of the Alzheimer's β-amyloid peptide (amino acids 29-40 and 29-42) are able to induce the fusion of lipid vesicles, suggesting that a direct interaction of the β-amyloid peptide with cell membranes might account for part of the cytotoxicity of the peptide. As apolipoprotein E polymorphism has been shown to influence the pathology of Alzheimer's disease, we examined the interaction between the apoE isoforms and the amyloid peptide. Our data show that only apoE2 and E3 are inhibitors of the amyloid peptide fusogenic and aggregational properties, whereas apoE4 has no effect. Stable complexes between apoE2 and E3 and the amyloid peptide could be detected by polyacrylamide gel electrophoresis. The C-terminal domain of the amyloid peptide seems therefore critical for the amyloid-apoE interaction. The apoE2 and E3 isoforms, might play a protective role against the formation of amyloid aggregates and/or against their interaction with cellular membranes, whereas apoE4 has no effect on these properties.
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Introduction

Alzheimer's disease (AD) affects an increasing number of the ageing population and is characterized by the formation in the brain of amyloid plaques, neurofibrillary tangles, and vascular and neuronal damages (1). The major component of amyloid plaques is a 39- to 42-residue peptide, named beta-amyloid peptide (Aβ), which is a proteolytic product of the amyloid precursor protein. The mechanism by which Aβ causes cell death and exerts its cytotoxic effect on neuronal cells has not been elucidated yet. The carboxy-terminal domain of Aβ seems critical for amyloid aggregation and for fibril formation (2) and recent studies have demonstrated that the soluble amyloid peptide might interact with cellular membranes. The Aβ peptide could thus be toxic via an oxidative mechanism, which is not mediated by a receptor pathway (3). Recent epidemiologic and biochemical observations suggest that apolipoprotein E (apoE), could play a role in the brain (4). The apoE genotype has recently been recognized as a susceptibility risk factor for AD (5). Three common apoE isoforms, E2, E3 and E4, differing by a single ARG-CYS substitution at position 112 or 158, have been identified in the human population (6). ApoE is associated with AD plaques *in vivo* and has been shown to bind to the circulating Aβ (1-40) peptide (5). We have recently demonstrated that the C-terminal domain of Aβ (e.g. residues 29-40 and 29-42) has fusogenic properties on large and small unilamellar lipid vesicles (7). We proposed that the C-terminal domain of Aβ might mediate the interaction of the amyloid peptide with cell membranes and the peptide toxicity. In view of the higher prevalence of the apo E4 isoform in patients with an early onset of Alzheimer's disease (AD) we analyzed the interaction between the three apoE isoforms and the C-terminal domain of the amyloid peptide. Our data show that the apoE3 and E2 isoforms can inhibit

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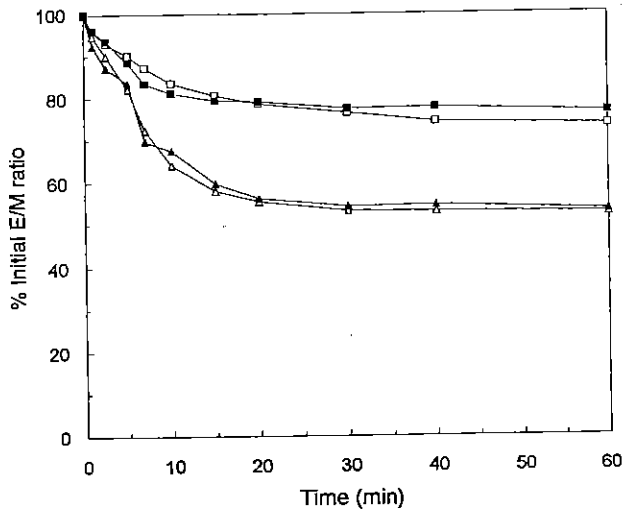
FIGURE 1
Effect of apoE isoforms on the lipid-mixing properties of the Aβ (29-40) amyloid peptide. Peptide aliquots were added to a mixture of labeled and unlaleled SUV's. The Pyrene-PC excimer/monomer ratio was monitored at room temperature, and is plotted, as a percentage of the initial value, *vs* time. Experiments were conducted with (D), the peptide alone, or in the presence of (o), recombinant apoE3, (n), recombinant apoE2, and (s), recombinant apoE4



liposomal fusion induced by the Aβ peptide, whereas apoE4 has no effect. We further demonstrate direct interactions between the apoE isoforms and the Aβ peptides, and propose that such interactions might account for the decreased amyloid toxicity associated with the apo E2 and E3 isoforms.

Methods

Experimental procedures

Peptides were synthesized by the standard F-moc solid-phase method, on an Applied Biosystems Model 431A peptide synthesizer. The purity and size of all peptides was verified by electronspray ionisation mass spectrometry using a Fisons/VG Platform (Manchester, UK) instrument. Recombinant apoE isoforms (apoE2, E3 and E4) were expressed in *E.Coli*, isolated and purified as previously described (8).

Fusion assays

Small unilamellar vesicles (SUV) were prepared from a mixture of phosphatidylcholine/phosphatidylethanolamine/phosphatidylserine/sphingomyelin/cholesterol (10:5:7.5:7.5:16, w/w), to mimick that of rat neuronal membranes. The lipid suspension was sonicated at 23°C, using a Branson sonifier, under nitrogen at 32 watts for 4 times 15 min and small vesicles were isolated on a Sepharose CL 4B column.

Fusion of pyrene-labeled SUV's together with unlabeled vesicles, at a 1/4, w/w ratio, was measured using a fluorescence probe dilution assay (9). Emission spectra were obtained on an Aminco SPF 500 spectrofluorimeter at 25°C. The pyrene Excimer/Monomer (E/M) ratio was calculated from the excimer and monomer fluorescence intensity at 475 and 398 nm, respectively, with an excitation wavelength of 346 nm.

The aggregation of amyloid peptide fragments was monitored as a function of time by measuring the turbidity of the solution at 400 nm.

Tryptophan fluorescence measurements

The wavelength shift of the fluorescence emission maximum of the apoE tryptophan residues was used to demonstrate a direct binding between the apoE protein and the synthetic amyloid peptides. Peptides were added to apoE at an apoE/peptide molar ratio of 1/100 and incubated at 25°C for 1 hour. Tryptophan fluorescence emission measurements were performed at 25°C on an Aminco SPF 500 spectrofluorimeter using an excitation wavelength of 295 nm.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed under non-reducing conditions. Protein bands were visualized after staining with Coomassie brilliant blue.

Results

Influence of the apoE isoforms on the lipid-mixing properties of the C-terminal β-amyloid peptides

The extent of lipid-mixing induced by 29-40 β-amyloid peptides, as a measure of their fusogenic activity, was tested with phospholipid/cholesterol SUV's, using a probe dilution assay (7, 10). In this assay, labeled and unlabeled vesicles were mixed in the presence of the peptides and of the apoE isoforms, and the ratio of the excimer to monomer intensity of the pyrene probe was monitored as a function of time (Fig. 1). Vesicle fusion results in a

decrease of the excimer intensity and an increase of the monomer intensity due to the dilution of the probe into the fused vesicles.

As shown in Figure 1, the fusogenic properties of the Aβ (29-40) peptide were significantly decreased by addition of apoE2 and apoE3, at a concentration of 140 nM, whereas the apoE4 isoform, added at the same concentration, had no effect on the decrease of the excimer/monomer intensity ratio induced by the Aβ (29-40) peptide. Similar results were obtained when apoE isoforms were incubated with the Aβ (29-42) amyloid peptide (data not shown).

The dependency of the decrease of the excimer/monomer intensity ratio upon apoE concentration, was investigated by adding increasing concentrations of the apoE isoforms to a fixed amount of the vesicles/Aβ (29-40) peptide mixture. The inhibitory activity of the apoE isoforms was compared by plotting the E/M ratio decrease after 10 min as a function of apoE concentration (Fig. 2). The data show that maximal inhibition of vesicle fusion by apoE was reached at apoE2 and E3 concentrations above 140 nM, corresponding to apoE/peptide molar ratios above 1/100. The selective inhibition by the apoE2 and E3 isoforms is confirmed by these results, as apoE4 had no detectable effect on vesicle fusion even at the highest concentration tested. When the Aβ (29-42) amyloid peptide was used to induce liposome fusion, same results were obtained (data not shown).

FIGURE 2

ApoE concentration effect on the fusogenic properties of the Aβ (29-40) amyloid peptide. The percentage of lipid-mixing of phospholipid/cholesterol SUV's induced by the Aβ (29-40) amyloid peptide after 10 min of reaction is plotted *vs* the concentration of apoE2 (o), apoE3 (u) and apoE4 (s). The fusion peptide concentration was 14 mM

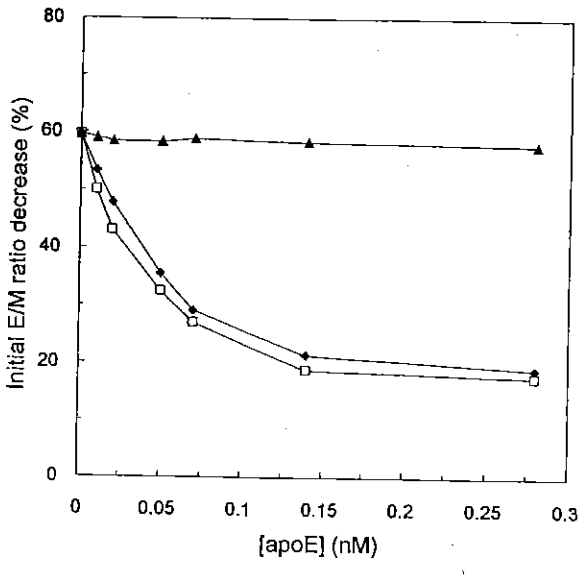
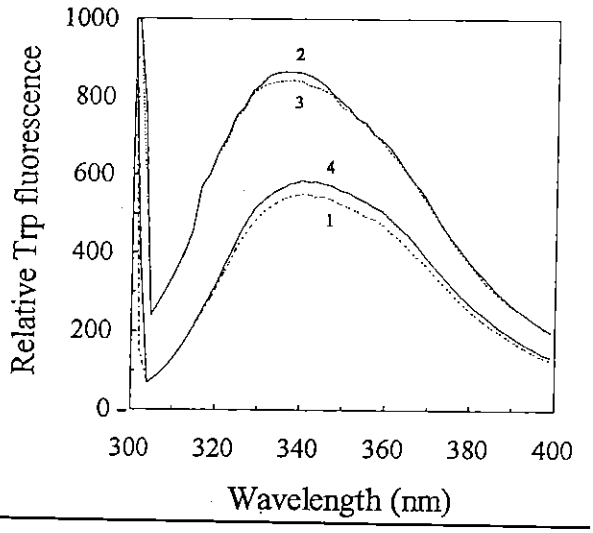


FIGURE 3

Effects of the Aβ (29-40) amyloid peptide on the tryptophan fluorescence emission spectra of the apoE isoforms. Fluorescence spectra of apoE3 alone (*line 1*) or of apoE2, apoE3 and apoE4 isoforms (*lines 2, 3* and *4*, respectively) incubated one hour at 25°C with the Aβ (29-40) amyloid peptide at an apoE/peptide molar ratio of 1/100 were recorded as described under *Experimental Procedures*



Visible absorbance measurements

The aggregation and/or fusion of lipid vesicles induced by tilted peptides can further be demonstrated by monitoring the absorbance increase at 405 nm for a mixture of vesicles and peptides. Addition of both Aβ (29-40) and Aβ (29-42) to phospholipid/cholesterol vesicles caused a rapid and extensive increase of the absorbance at 405 nm, indicative of an increase of the vesicle size (data not shown). In the presence of apoE2 and apoE3 at a apoE/peptide molar ratio of 1/100, the final absorbance increase was more limited, while the addition of apoE4 had no effect on the vesicle aggregation induced by the β-amyloid peptides (data not shown). These results support a specific interaction between the β-amyloid peptides and apoE2 and E3 and suggest that apoE4 cannot adopt the conformation required to interact with the amyloid peptides.

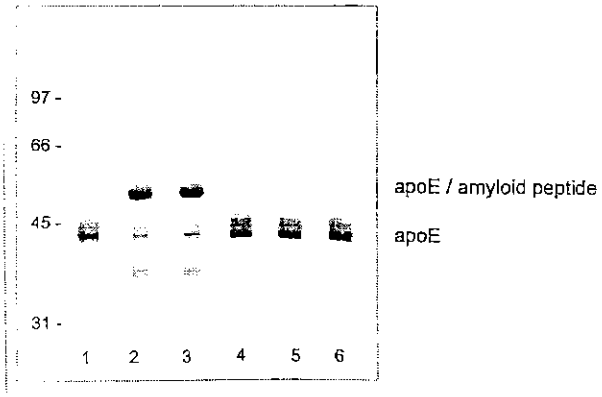
Monitoring of apoE/amyloid peptide complex formation

In order to demonstrate a direct interaction between apoE and the amyloid fusion peptide, we investigated the effect of the peptide incubation with apoE on the intrinsic fluorescence of the apoE tryptophan residues. In the absence of the amyloid peptide, the maximal tryptophan emission fluorescence of apoE3, E2 and E4 is at 343 nm, (8, 11). After incubation with the Aβ (29-40) peptide, the maximal fluorescence emission wavelength of apoE2 and E3 incubated is blue-shifted to 336 nm (Figure 3, curves 2 and 3) relative to apoE alone (Figure 3, curve 1), and the fluorescence yield is increased. This is indicative of an increased shielding of the apoE2 and E3 tryptophan residues, due to a direct interaction with the β-amyloid fusion peptide. In contrast, the emission spectra of apoE4 remains identical in the presence or absence of the amyloid peptide, supporting the lack of interaction suggested by above data.

To confirm the specific nature of the interaction between apoE2 and E3 and the amyloid fusion peptide, we visualized the formation of apoE/amyloid peptide complexes on a 10% SDS-PAGE. As shown in Figure 4, recombinant apoE migrates as a single polypeptide band of 40.5 kDa when the electrophoresis is performed under non-denaturating conditions. After one hour incubation with the peptide at 25°C, a new protein band appeared with an apparent molecular weight of 51-52 kDa corresponding to a stable apoE/Aβ (29-40) complex containing 4 to 5 peptide chains. This complex was detected only when apoE2 and E3 were incubated with the Aβ (29-40) peptide, whereas no complex was formed with the apoE4 isoform.

FIGURE 4

Monitoring of apoE/Aβ (29-40) complex formation by 10% SDS-PAGE. ApoE isoforms were incubated separately with the Aβ (29-40) amyloid peptide for one hour at 25°C and at an apoE/peptide molar ratio of 1/100. Proteins were then separated by SDS-PAGE under non-denaturating conditions and protein bands were visualized after Coomassie blue staining. ApoE4, E3 and E2 incubated with the Aβ (29-40) (*lanes 1, 2,* and *3*), and apoE4, E3 and E2 incubated alone (*lanes 4, 5* and *6*)



Discussion

The results obtained from the study of the C-terminal fragments of the Aβ peptide suggest that these peptides have fusogenic properties similar to those of viral proteins fusion peptides (7). We therefore proposed that these fusogenic properties could mediate the direct interaction between the amyloid peptide and cellular membranes, in agreement with an hypothesis previously proposed (12). The present data demonstrate that the fusogenic properties of the C-terminal fragments of the Aβ peptide are partially inhibited by low concentrations of the apoE2 and E3 isoforms, while the apoE4 isoform has no effect on the peptide-induced vesicle fusion. Accordingly, a rapid and apoE isoform-specific interaction can take place with the amyloid fragments at physiological concentrations of apoE, as this protein is produced in the human brain at a concentration close to those used here. At low concentrations, apoE can decrease the neurotoxicity of the β-amyloid protein, due to inhibition of either amyloid plaque formation or interaction between the amyloid peptide and cellular membranes. The results presented here suggest the existence of a specific conformational domain on the apoE2 and E3 isoforms, able to bind the C-terminal fragment of the amyloid peptide and thereby to inhibit peptide aggregation and peptide interaction with phospholipids. The increased risk factor associated with the apoE4 isoform in the AD pathology, could thus be explained by the lack of interaction between this isoform and the C-terminal amyloid fragments, as previously proposed (4, 13). Moreover, the specific interaction of the C-terminal fragments of the β-amyloid protein with the apoE2 and E3 isoforms could explain the potential protective role of these apolipoproteins in the etiology of AD, by preventing not only the aggregation of the amyloid peptide but also its interaction with cells. Our results are consistent with the previous suggestions that the amyloid peptides interact with the more hydrophobic C-terminal domain of the apoE2 and E3 isoforms (4, 5). The lack of interaction between the apoE4 isoform and the amyloid fragments, described here, suggests that the Cys-Arg mutation at position 112 in the apoE4 isoform decreases the ability of apoE4

to bind the Aβ peptides, thus emphasizing the cooperativity between the N- and the C-terminal domains of apoE. Taken together with the observations on the interactions between apo AI and the SIV fusion peptide (14), our results suggest that specific interactions and complementarity exist between some apolipoproteins and tilted fusogenic peptides. Apolipoproteins might therefore play a protective role against several membrane destabilizing events besides their lipid transport properties in plasma.

Acknowledgements

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