INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in adults. It determines a progressive loss of intellectual function and appears both sporadically and in an autosomal dominant (familial) form. Presently there is no treatment for AD.

An early manifestation of the disease is the deposition of amyloid as insoluble fibrous masses in extracellular senile plaques. The main component of amyloid is a 4.3 kDa hydrophobic peptide, amyloid beta-peptide (Aβ), that is encoded within the larger amyloid precursor protein (APP) gene, localized on chromosome 21 (Sisodia and Price, 1995). In addition to amyloid deposits, Aβ is also found in a soluble form (sAβ) in cerebrospinal fluid and plasma from normal individuals and AD patients (Wisniewski et al., 1994b). The mechanism by which amyloid deposits are associated with the dementia in AD is unknown, although controversial reports have suggested that when Aβ aggregates into amyloid fibrils, it becomes neurotoxic leading to cell death (Lorenzo and Yankner, 1994; Pike et al., 1993).

Peptides containing the sequence 1-40 or 1-42 of Aβ and shorter derivatives can form amyloid-like fibrils in vitro. It has been suggested that Aβ fibrillogenesis is modulated not only by concentration, pH and clearance rate, but also by the interaction with several Aβ-binding proteins, including: apolipoproteins (apo) E and J, α1-antichymotrypsin, serum amyloid P, and heparan sulfate proteoglycans (Wisniewski et al., 1994b). Immunohistochemical analysis indicated that these proteins are co-deposited with
amyloid in senile plaques and *in vitro* studies showed that Aβ-binding proteins affect the fibrillogenesis rate. Whether they are inert bystanders or participating actively in the amyloidogenesis process is unknown. Several lines of evidence suggest that at least the two apolipoproteins interact *in vivo* with amyloid or sAβ. The high-affinity interaction (Kd = 2 nM) between Aβ and apoJ, the identification of the complex apoJ-sAβ in CSF, the co-localization of sAβ with apoJ-containing high density plasma lipoproteins, the participation of apoJ in the transport of Aβ through the blood-brain barrier and the demonstration that the interaction of Aβ with apoJ prevents peptide aggregation and degradation *in vitro* (Matsubara et al., 1996), indicate that this protein plays an important role as a physiological carrier for sAβ. On the other hand, apoE also binds with high affinity to Aβ (Kd = 10-20 nM), but this interaction appears to induce a change in Aβ conformation and increases the ability of the peptide to form amyloid fibrils *in vitro*, under certain conditions (Wisniewski et al., 1994a; Ma et al., 1994; Soto et al., 1996). Genetic studies have shown a close association between the allele ε4 of apoE and both sporadic and familial late-onset AD (Corder et al., 1993). In addition, it has been shown that apoE and C-terminal fragments derived from it are tightly complexed with Aβ fibrils in AD amyloid deposits (Naslund et al., 1995; Wisniewski et al., 1995). The wide association of apoE with clinically and biochemically distinct types of amyloid deposits had led to the suggestion that apoE may act as pathological chaperone in amyloidogenesis, binding to the amyloid precursors and modulating fibril formation, possibly by inducing or stabilizing β-pleated sheet conformation (Wisniewski and Frangione, 1992).

Several studies have shown that amyloid formation is driven by hydrophobic interactions and is dependent on conformational changes of amyloid precursors. The influence of hydrophobicity and β-sheet secondary structure on amyloid formation is also evident by comparison of the sequence of other amyloidogenic proteins. It is likely that hydrophobicity facilitates monomeric interaction and that β-sheet content drives this interaction to β-sheet oligomers and amyloid fibrils.

In this study, we designed inhibitors of fibrillogenesis based on the hypothesis that amyloid formation could be inhibited by peptides homologous to Aβ, with a similar degree of hydrophobicity but with a very low propensity to adopt a β-sheet conformation. The aim was to design a short Aβ homologous peptide with sufficient similarity to interact with Aβ and with the ability to block the formation of intermolecular β-pleated sheets and, therefore, the formation of amyloid fibrils.

**Design of inhibitor peptides**

We focused on the central hydrophobic region within the N-terminal domain of Aβ, amino acids 17-21 (LVFFA), as a model for our inhibitor peptide (Fig. 1A). Proline residues were introduced in the inhibitor peptide in order to block β-sheet structure and charged residues were added at the ends of the