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Alzheimer's Disease-Associated Presenilins 1 and 2: Accelerated Amyloid Fibril Formation of Mutant 410 Cys→Tyr and 141 Asn→Ile Peptides

C. P. J. Maury,* E-L. Nurmiaho-Lassila,† and M. Liljeström*

**Department of Medicine, University of Helsinki, Kasarminkatu 11-13, FIN-00130 Helsinki, Finland; and †Department of Biosciences, Division of General Microbiology, University of Helsinki, Helsinki, Finland*

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Mutations in the presenilin 1 gene on chromosome 14 and in the related gene on chromosome 1 have been identified in individuals with early-onset familial Alzheimer's disease. The functions of the presenilin gene products as well as the relation of the presenilin mutations to amyloidogenesis are unclear. Here we show that peptides homologous to two disease-associated mutant forms of presenilin 1 and 2, respectively, show highly increased amyloid fibril formation as compared with the wild-type peptide homologues. The 410 Cys →Tyr (S-182) 14-residue peptide and the 141 Asn→Ile (E5-1) 15-residue peptide spontaneously assemble to fibrils of 7 to 9 nm width with strong Congophilic characteristics. Thioflavine-T fluorometry reveals a 7- to 18-fold higher rate of amyloid fibril formation of the mutant peptides as compared with the corresponding wild-type homologues. The results provide new insights into the mechanisms by which presenilin mutations lead to Alzheimer-type neuropathology: missense mutations in the presenilin genes may create products that are intrinsically highly amyloidogenic and directly involved in pathogenesis.

Missense mutations in the presenilin gene on chromosome 14 (PS-1) and in the related gene on chromosome 1 (PS-2) have recently been identified in a high portion of individuals with early-onset familial Alzheimer's disease (FAD,refs.1-8). The function(s) of the presenilin gene products, S-182 protein of SP-1, and E5-1 of SP-2, respectively, as well as the relation of the presenilin mutations to amyloidogenesis in FAD are unclear. According to the amyloid theory of Alzheimer's disease (9,10) altered amyloid β-precursor protein (AβPP) metabolism is a central event in the process leading to the generation of fibrillar amyloid β protein (Aβ). The AβPP/Aβ system is considered a final common pathway for amyloid deposition in the brain. In conformity with this theory, evidence has been presented suggesting that mutations in PS-1 may act by influencing AβPP processing resulting in increased Aβ production (11). However, the possibility exists that the presenilin mutations may act in a more direct way by inducing conformational changes in protein structure that are amyloidogenic themselves.

To address this hypothesis, we synthesized peptides homologous to wild-type (residues 400-413) or mutant (residues 400-413 with a tyrosine-for-cysteine substitution at position 410) S-182 protein corresponding to the naturally occurring pathogenetic FAD3 type PS-1 mutation (1,4), as well as wild-type (residues 129-143) or mutant (residues 129-143 with an isoleucine-for asparagine substitution at position 141) E5-1 protein corresponding to the naturally occurring pathogenetic Volga-German type PS-2 mutation (2,6) and tested their amyloido-genicity. *In vitro* amyloid fibril formation was monitored by Congo-red staining and polarization microscopy, by electron microscopy and quantitative fluorometry (12-14).

METHODS

Synthetic peptides homologous to wild-type or mutant S-182 and E5-1 were synthesized (Genosys Biotechnologies Inc.). The C-terminal carboxyl of the peptides was blocked by amidation. The purity of all peptides was >95% as judged by high-pressure liquid chromatography on a Vydac RP C-8 column. The molecular weights were confirmed by mass spectrometry. The peptides were as follows (one-letter-code designation for the amino acids): ASGDWNTTIACFVA (residues 400-413 of wild-type S-182), ASGDWNTTIAYFVA (residues 400-413 of mutant S-182), PSVGQRLLNSVLNTL (residues 129-143 of wild-type E5-1), and PSVGQRLLNSVLITL (residues 129-143 of mutant E5-1). For *in vitro* amyloid formation the peptides were dissolved in water at a concentration of 5mg/ml and analyzed for Congo-red positivity and birefringence in polarized light after 15 min and over-night incubation at room temperature (14). Quantitation of amyloid fibril formation was monitored by fluorometry as described (13,14). The method is based on the fluorescence of thioflavine-T in the presence of amyloid fibrils at the excitation and emis-

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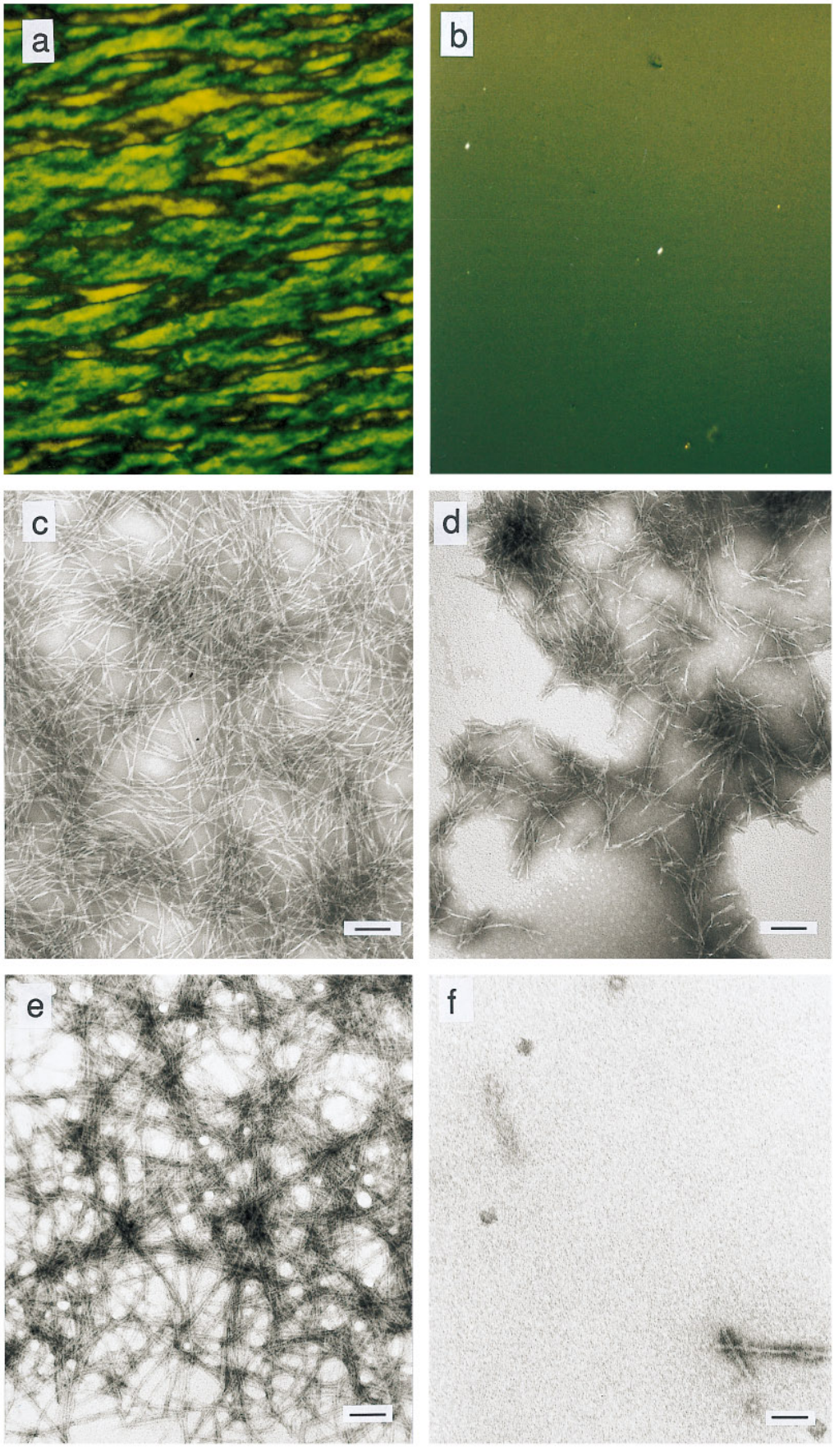
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sion maxima of 446nm and 490nm, respectively. We used 50mM Glycine-NaOH, 5μM thioflavine-T, pH 8.5, as assay buffer, an assay time of 1 s, and a Perkin-Elmer Luminescence Spectrometer LS 30. Aliquots of 0,5,10,15, and 25μl of the synthetic S-182 (0.5mg/ml) and E5-1 (5mg/ml) peptides were diluted in the assay buffer (final volume 640μl) and fluorescence at 490 nm (relative units) was measured. Negative staining electron microscopy was carried out on peptide samples that were dissolved in water at a concentration of 5mg/ml, incubated at room temperature for 15 min, and frozen at −20°C. After thawing the samples were diluted 1:1 in water and applied to a copper grid coated with pioloform and carbon, and stained with phosphotungstic acid adjusted to pH 6.5 with potassium hydroxide. The grids were examined with a JEOL-JEM 100CX or 1200EX transmission electron microscope at an operating voltage of 60 kV.

RESULTS

The mutant S-182 and E5-1 peptides formed aggregates that were Congo-red positive and showed the typical green birefringence of amyloid in polarized light (Fig. 1a). In electron microscopy, amyloid-like fibrils of 7 to 9 nm in width were seen (Fig.1c,e). In contrast, the peptide assemblies formed under the same conditions from the corresponding wild-type peptides were either only slightly positive (S-182) or negative (E5-1,Fig.1b) in the Congo-red test; ultrastructurally, either relatively short fibrils (S-182, Fig.1d) or mainly nonfibrillar deposits (E5-1,Fig.1f) were detected. Quantitative thioflavine-T based fluorometry revealed a 7- to 18-fold higher rate of amyloid fibril formation by the mutant S-182 and E5-1 peptides as compared with the corresponding wild-type peptides (Fig.2). For fibrillogenesis, the mutant E5-1 peptide required considerably higher peptide concentrations than did the mutant S-182 peptide (Fig.2).

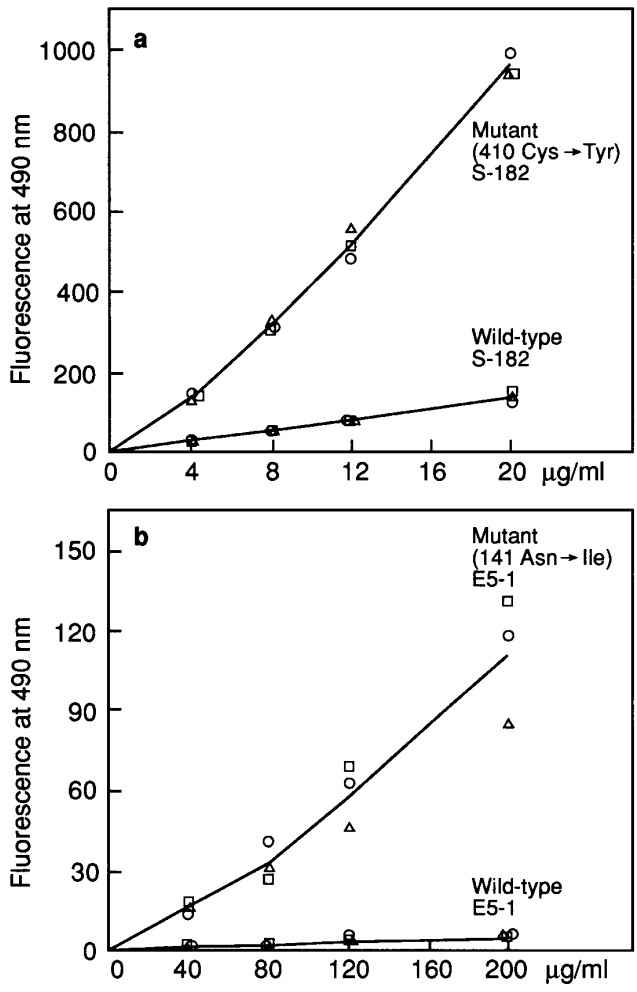

FIG. 2. Amyloid fibril formation of synthetic mutant and wild-type presenilin homologs by thioflavine-T based quantitative fluorometry. The fluorescence (relative units) at the emission maximum of 490nm was measured. The mean and individual (symbols) values of measurements carried out on three consecutive days are shown.

DISCUSSION

The present results provide new insights into the possible mechanisms by which presenilin mutations lead to Alzheimer-type neurodegeneration. Both the 410 Cys→Tyr substitution in S-182, and the 141 Asn→Ile substitution in E5-1, respectively, lead to conformational changes in peptide structure that make them markedly more amyloidogenic than the corresponding wild-type peptides. Deposition of presenilin-derived fibrils in the neuronal cell population, the primary site of presenilin expression in the brain (15), could be associated with neurotoxicity, as has been shown for fibrillar forms of Aβ (16). If this view is correct, one would expect to find presenilin-derived fibrils in the senile, amyloid plaques of FAD. Indeed, Wisniewski et al. (17) recently demonstrated the presence of S-182 in chromosome 14-linked and other types of Alzheimer's disease.

The present findings are not in contradiction to previous demonstrations of increased Aβ production and Aβ phenotype in presenilin-linked FAD (11). The deposition of presenilin fibrils could merely be an initiating

FIG. 1. Light and electron micrographs of assemblies of mutant (left panel) and wild-type (right panel) presenilin peptides. a,Aggregates of the mutant (141 Asn→Ile) E5-1 peptide stained with Congo-red and viewed under polarized light show typical green birefringence of amyloid. b,Corresponding wild-type E5-1 peptide aggregates are Congo-red negative and show no green birefringence in polarized light. c,Electron micrograph of the negatively stained aggregates of mutant (410 Cys→Tyr) S-182 peptide shows amyloid-like fibrils of 7 to 9 nm in width. d,the corresponding wild-type S-182 peptide assemblies consist of atypical filaments. e,Amyloid-like fibrils produced by mutant (141 Asn→Ile) E5-1 peptides, and f,corresponding wild-type E5-1 peptides form assemblies with mainly nonfibrillar morphology. The scale bar is equal to 100nm.

factor in the AβPP/Aβ cascade leading the formation of β-amyloid fibrils (9,10), the major component of the amyloid deposits. The amyloidogenicity of a mutant presenilin could also interfere with its normal function(s) suggested to involve, e.g., AβPP transport and processing, axonal transport and receptor recycling (1).

It has become increasingly evident that mutation-induced amyloidogenic conformational change in protein structure is an important pathogenetic mechanism in hereditary amyloidosis, including cerebral amyloid angiopathy (18) Finnish and Danish type cranial amyloid neuropathy (14,19,20). To date, 25 pathogenetic mutations in the PS-1 gene and two in the PS-2 gene have been described (1-6). These mutations have all involved single amino acid substitutions. Further studies are needed to clarify how many of these mutations result in the creation of an amyloidogenic conformation and to define the role of this type of mechanism in the pathogenesis of presenilin-linked FAD.

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