Proteolytic processing of presenilin-1 (PS-1) is not associated with Alzheimer's disease with or without *PS-1* mutations

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Received 15 October 1997

Abstract Cerebral presenilin-1 protein (PS-1) is normally composed of the amino-terminal fragment (NTF) with M_r 28 kDa and the carboxy-terminal fragment (CTF) with 18 kDa. We analyzed human PS-1 in brains with early-onset familial Alzheimer's disease (FAD) with and without PS-1 mutations to study whether mutated PS-1 was abnormally metabolized. Cerebral PS-1 were found to be cleaved into two fragments of NTF and CTF independently of the occurrence of PS-1 mutation in human brains. A small portion of PS-1 was recently found to suffer another processing by caspase-3, an apoptosis-related cysteine protease. In contrast to the recent finding that the Volga-German mutation on presenilin-2 (PS-2) affects the increasing caspase-3 PS-2 fragment, the PS-1 mutation did not cause a significant change in PS-1 fragmentation. We conclude that PS-1 fragmentation and other (probably caspase-3-mediated) digestion following apoptosis occur independently of *PS-1* mutations.
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Key words: Alzheimer's disease; Presenilin; Mutation; Caspase-3; CPP32

1. Introduction

Alzheimer's disease (AD) is a progressive intellectual failure in elderly people. The causal genes have been identified as *presenilin 1* (*PS-1*) on chromosome 14q24.3 [1,2], *presenilin 2* (*PS-2*) on chromosome 1q42.1 [3,4] and *APP* on chromosome 21 [5,6] while the *apolipoprotein E* allele e4 has been established to be a risk factor located on chromosome 19 [7]. *Presenilin* genes are members of a novel highly conserved family [8] whose function is not fully understood, and are expressed widely not only in the brain but also in other tissues. In all tissues, PS-1 is found to be essentially cleaved into two frag-

ments: the amino-terminal fragment (NTF) with M_r 28 kDa and the carboxy-terminal fragment (CTF) with M_r 18 kDa [9–12]. This fragmentation was claimed to be affected by the PS-1 mutation [13,14]. However, there are reports describing the different effects of the *PS-1* mutation on PS-1 cleavage in cultured cells, animals and human brain [9,15,16]. More evidence should accumulate for PS-1 in human tissues to obtain concrete conclusions. Here, we studied human brains with and without PS-1 mutations to address this question and provide more detailed information on PS-1 processing in brain.

2. Materials and methods

2.1. Preparation of monoclonal and polyclonal antibodies
Polyclonal and monoclonal antibodies were against NTF and CTF of PS-1, respectively. To prepare NTF antibodies, mice and rabbits were immunized with the synthetic peptide (DNRER-QEHNDRRSLGHPEPLSNGRPQ) to produce a polyclonal antibody (AD3N) and a monoclonal antibody (PSN2) [17]. To prepare CTF antibodies, mice and rabbits were immunized with the synthetic peptide (MAEGDPEAQRRVSKNSKYNAESTERESQDTV) to produce polyclonal antibodies (AD3L and mAD3L), respectively [17].

2.2. Preparation of PS-1 from human brains
The brain tissues of subjects with early-onset FAD analyzed here carried the *PS-1* mutations of Ile[143]Thr, Met[146]Leu, His[163]Arg, Ala[246]Glu and Cys[410]Tyr [1,4,18]. Brain tissues were homogenized with 50 mM Tris-Cl buffer (pH7.6), 150 mM NaCl containing 0.1 mM diisopropyl fluoro-phosphate, 0.5 mM phenylmethylsulfonyl fluoride, 1 µg/ml 1-chloro-3-tosylamino-7-amino-2-heptanone, 1 µg/ml antipain, 0.1 µg/ml pepstatin, 1 µg/ml leupeptin (TS buffer) followed by ultracentrifugation at 100000×g for 20 min at 4°C. The supernatant was saved as the soluble fraction. The resultant pellet was sequentially extracted with 1% Triton X-100 in TS buffer (Tx buffer), 1% Triton X-100, 0.5% deoxycholic acid, 0.1% SDS in TS buffer (RIPA buffer) and 2% SDS in TS buffer. The membrane fraction extracted with Tx buffer was further applied onto affinity column coupled to PSN2 or AD3L, specifically eluted with 0.1 M glycine-HCl (pH 2.5) and immediately neutralized. These monoclonal and polyclonal antibodies (PSN2, AD3N and AD3L) were purified by affinity chromatography coupled with the relevant antigen synthetic peptides.

2.3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting
SDS-PAGE was carried under reducing conditions, and proteins in

gels were transferred on PVDF membrane (MSI). The membrane was blocked with 3% gelatin, incubated with PSN2 followed by the horse-radish peroxidase (HRP)-conjugated second antibody. The color was developed with 4-chloro-1-naphthol.

2.4. Quantitation of protein concentration

Protein concentration was determined by measuring the absorption at 562 nm using the BCA protein assay kit (Pierce, IL).

3. Results and discussion

3.1. Gel electrophoretic analysis of human brain PS-1

To get a specific and high recovery of PS-1 from human brains, we used the two-step detection method to identify PS-1. We used the two antibodies for immunoprecipitation as the first step and Western blotting as the second step. AD3N and PSN2 were used to detect NTF while AD3L and mAD3L were used to detect CTF. As seen in Fig. 1a, NTF in aged normal brains was found predominantly in the membrane fraction that was obtained with Tx buffer. CTF was found both in the membrane and partly in the cytoskeleton fractions. This result suggests two possibilities. CTF was some-what insoluble as compared with NTF. Alternatively, a por-tion of CTF was associated with filamentous cytoskeletons such as neurofilaments. Similar results were obtained in PS-1 derived from brains with sporadic AD (Fig. 1b) and early-onset familial AD with PS-1 mutation (His[163]Arg) (Fig. 1c). These data could provide one clear conclusion that the PS-1 mutation (His[163]Arg) did not affect PS-1 processing to sup-port another FAD case with the PS-1 mutation (Gly[384]Ala)
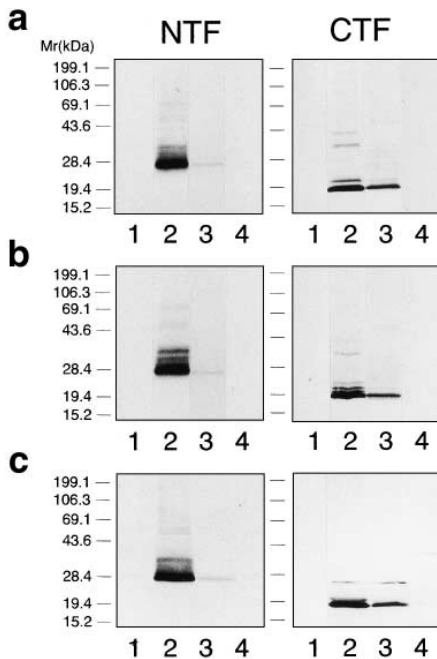
Fig. 1. PS-1 NH2 fragment (NTF) and COOH fragment (CTF) in subcellular fraction of human brains. Brain cortices were homogen-ized and separated into four fractions (see Section 2). Each fraction was immunoprecipitated with affinity-purified AD3N or AD3L fol-lowed by gel electrophoresis/Western blotting with PSN2 or mAD3L, respectively. a: aged normal, b: sporadic AD and c: fa-milial AD with PS-1 mutation (His[163]Arg). Lane 1: supernatant fraction extracted with TS buffer; lane 2: supernatant fraction ex-tracted with 1% Triton X-100; lane 3: supernatant fraction ex-tracted with RIPA buffer; lane 4: RIPA buffer precipitate fraction.
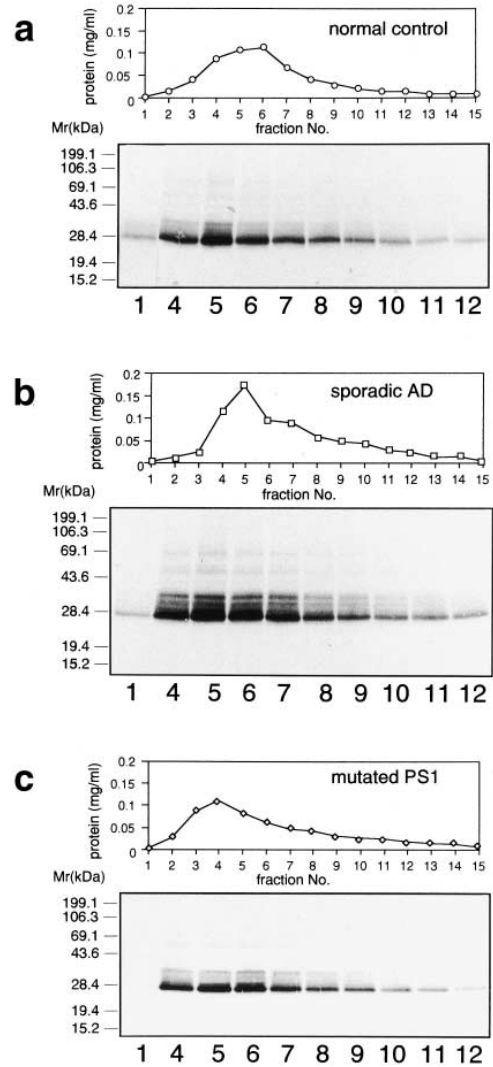

Fig. 2. Immunoaffinity column chromatography of PS-1 fragments (NTF) in normal, sporadic and familial AD brains. Triton X-100 supernatant fractions from brains of (a) aged normal subject (0.4 g), sporadic AD (0.5 g) and familial AD with PS-1 mutation (His[163]Arg) (0.25 g) were applied onto an affinity-purified AD3N-coupled affinity column. The PS-1-containing fraction was eluted with 0.1 M glycine-HCl, pH 2.5 and immunoblotted with PSN2.

[16] unlike in previous studies performed in cultured cells [13,14].

We next attempted to quantitatively obtain PS-1 fragments. For this purpose, we prepared an immunoaffinity column to obtain the PS-1 fragment. Synthetic peptides used as antigens (see Section 2) were coupled with Sepharose 4B. Antibodies were applied onto this affinity column followed by washing with PBS and eluted with 0.1 M glycine-HCl, pH 2.5. The eluent was immediately neutralized with 2 M Trizma. Purified antibodies were then coupled to Sepharose 4B. The membrane fraction from human brains was applied onto a purified anti-body-coupled affinity column and eluted (Fig. 2). As seen in protein concentrations in Fig. 2, the amounts of PS-1 frag-ment were different. However, the molecular compositions of PS-1 were essentially identical as seen in the result obtained from the immunoprecipitation experiment (Fig. 1).
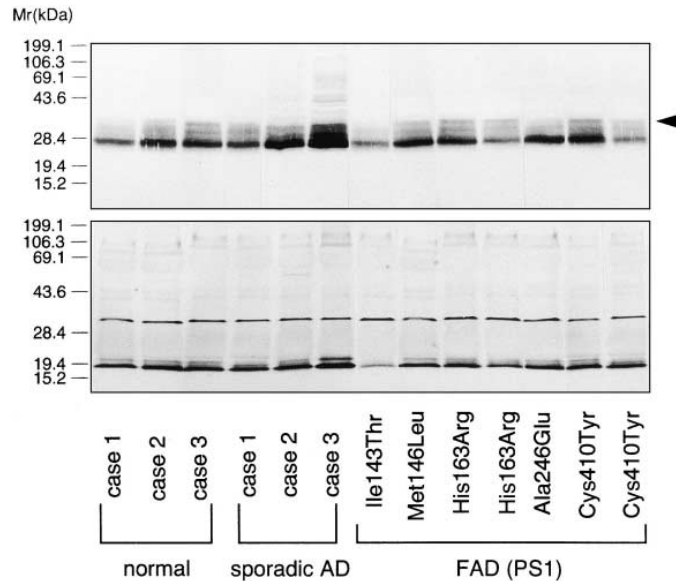
ing into consideration experimental deviation, the present data may suggest that the 34 kDa NTF (caspase-3 fragment) of PS-1 was not tightly associated with PS-1 mutations. We could not find a significant difference in the ratio of this 34 kDa NTF/28 kDa NTF between sporadic AD and familial AD bearing PS-1 mutations (data not shown). In spite of the present results on PS-1, an involvement of caspase-3 in AD pathogenesis may be important to understand the pathogenesis of FAD with *PS-2* mutations. Caspase-3 is widely believed to play a role in mammalian apoptosis (programmed cell death), an evolutionarily conserved form of cellular suicide. If this enzyme is related to AD pathogenesis, it is very interesting because it gives hope for drug treatment for AD patients. A recent report is suggestive of the involvement of caspase-3 in the pathological metabolism of PS-2 [19] but this still needs to be studied more. It is worth noting that the expression amounts of presenilin (PS-1 and PS-2) themselves could be a potent factor to influence the processing of presenilin as previously described [9]. Therefore, the site-specific effects of the mutations seen in Fig. 4 might come from the different amounts of mutated *PS-1* expressed in human brains. These results on the *PS-1* mutations are different from previous ones in cultured cells or transgenic mice [9,13,14]. We believe that both their data [9,13,14] and our data [15,16] are true since the apparent discrepancy comes from the physiological expression or experimental expression. Thus, the level of *PS-1* gene expression is a critical issue if we consider the possibility that the full-sized PS-1 plays a different role from PS-1 fragments or the gene product is in competitive balance with other molecules. It is suggested that the *PS-1* mutation be considered at least in the relation among PS-1, PS-2, and βAPP but it has no influence on the conventional and 34 kDa NTF-generating (caspase-3) cleavage of mutated PS-1 at least in human brains.

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