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Accurate Determination of Carboxyl-Terminal Fragment of Presenilin 1 in Various Tissues from Rat and Cell Lines

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Presenilin 1 (PS1) has been identified as a causative gene for the early-onset of familial Alzheimer's disease, and it is mainly localized in the endoplasmic reticulum and the Golgi membrane as a multiple membrane-spanning protein. In the cell, PS1 is proteolytically processed to a 30-kDa N-terminal fragment and a 20-kDa C-terminal fragment (CTF), both of which exist as a stable high-molecular-weight protein complex, together with other components of γ-secretase. However, as there has been no report about the precise amount of PS1 expressed in mammalian tissues, the aim of this study was to quantitatively determine PS1–CTF amounts in various tissues such as liver, kidney, brain and heart of rat by western blotting using a [^{35}S]-methionine-labelled PS1-CTF as a standard synthesized in a wheat germ cell-free protein synthesizing system. PS1–CTF contents in kidney, liver, brain and heart were 17.0, 6.6, 6.4 and 0.2 fmol/mg protein, respectively. PS1–CTF contents were also determined in cultured cell lines such as HeLa, HEK293 and COS-1.

Key words: Alzheimer's disease, cell-free protein synthesis, presenilin 1, quantitative analysis, western blotting.

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; APP, amyloid β precursor protein; CTF, C-terminal fragment; FAD, early-onset familial Alzheimer's disease; GST, glutathione S-transferase; MBP, maltose-binding protein; NTF, N-terminal fragment; PS1, presenilin 1; PMSF, phenylmethylsulphonyl fluoride; PVDF, polyvinylidene difluoride; TL-PS1-CTF, PS1-CTF synthesized *in vitro*.

Alzheimer's disease (AD) is caused by the deposition of amyloid β(Aβ), which is proteolytically produced from the amyloid β precursor protein (APP) through two sequential cleavages by β- and γ-secretase, which produce the N- and C-termini of Aβ, respectively. The β-site APP cleaving enzyme (BACE) was identified as β-secretase (*1*), and presenilin 1(PS1) identified as a causative gene for early-onset familial AD (FAD) (*2*, *3*). PS1 is required for the γ-secretase activity (*4*, *5*), and is suggested to be a component of the γ-secretase itself or as one of its regulating factors. PS1 is encoded on chromosome 14 (*2*), mainly localized in endoplasmic reticulum (ER) and the Golgi membrane as a multiple membrane-spanning protein (*6–11*), proteolytically processed to an N-terminal fragment (NTF) and a C-terminal fragment (CTF) with a molecular weight of ~30 kDa and 20 kDa, respectively (*12*, *13*). Both fragments of PS1 are highly stabilized by forming a high-molecular-weight complex (*14*) together with Nicastrin, Aph1 and Pen2 (*15–19*). Recently, Sato *et al*. (*20*) isolated functional γ-secretase complexes by immunoprecipitation of solubilized membrane fraction from cultured cells, and determined the relative ratio of four components of γ-secretase and demonstrated the stoichiometry of the γ-components presenilin:Pen-2: nicastrin:Aph-1 is 1:1:1:1.

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As mentioned earlier, since 1995, the study on PS1 has mainly focused on its molecular characterization and its function. PS1 mRNA has been shown to be expressed in all human (*2*) and mouse tissues (*21*). Though PS1 protein expression in human and mouse tissues was investigated by qualitative analysis (*12*, *21*), quantitative analysis of PS1 protein in various mammalian tissues has never been reported. PS1 amount in tissues is extremely low and it is very difficult to purify PS1 from mammalian tissues. As PS1 is an integral membrane protein, it is generally difficult to obtain PS1 synthesized in *Escherichia coli* that can be used as the standard protein in western blotting analysis for the determination of protein amount. In this study we determined the accurate amount of PS1–CTF in various tissues from rat and cultured cell lines conventionally used by western blotting analysis in which PS1–CTF synthesized *in vitro* using a wheat-germ extract was used as a standard protein.

MATERIALS AND METHODS

Preparation of Various Tissue Homogenates from Rat—Two male Sprague-Dawley rats weighing ~300 g were killed under anaesthesia with ethyl ether. Excised liver (5 g/21.44 g), kidney (4.47 g), brain (3.37 g) and heart (2.40 g) were homogenized in 5 vol of buffer A [0.25 M sucrose, 10 mM potassium HEPES (pH 7.5), 1 mM EDTA, 0.1% ethanol, 5 μM pepstatin, 5 μM chymostatin, 5 μM antipain, 5 μM leupeptin and 0.2 mM phenylmethylsulphonyl fluoride] by five strokes of an Elvehjem–Potter homogenizer at 4°C, respectively. Protein concentration

was determined by Lowry's method using bovine serum albumin as a standard protein (*22*). PS1–CTF in the various tissue homogenates was separated on 12.5% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) and visualized by western blotting.

Plasmid Construction—Human PS1–CTF and M457L PS1–CTF comprising the 299–467 amino acid (aa) region of human PS1 with an initiation methionine, were amplified from pcDNA-PS1 (*7*) by the polymerase chain reaction (PCR) method. The primer sequences used for PCR were as follows: 5′-tggaattctccaccatggcagaaggaga cccggaa-3′ as sense primer, 5′-tgctcgagtcagatataaaattgatg gaa-3′ or 5′-cactcgagtcagatataaaattgatggaatgctaattggtcca gaaaaggctgtacaagataatc-3′ as antisense primers, respectively. The PCR fragments were digested with EcoR1/Xho1 and inserted into the EcoR1/Xho1 sites of pcDNA3.1+ vector (Invitrogen™), respectively. The DNA sequences of construct were verified using the DYEnamic ET Terminator cycle sequencing kit (GE Healthcare) and MegaBACE 1000 DNA Analysis System (GE Healthcare).

In Vitro Transcription and Translation—The plasmids coding for the wild-type PS1-CTF and M457L PS1-CTF [both polypeptides consisting 169 aa of PS1–CTF (299–467 aa) plus an additional initiator methionine residue at the N-terminus] were linearized with Xba1 and were transcribed *in vitro* using T7 RNA polymerase according to the manufacturer's instructions (Stratagene, USA). In a typical experiment, 1 μg plasmid DNA was transcribed in 25 μl of transcription mixture. For the synthesis of [^{35}S]-labelled wild-type- or M457L- PS1-CTF, each transcript was translated using wheat-germ extract (kindly provided by Dr. T. Endo: Nagoya Univ., Japan). Each 25 μl protein synthesizing system (pH 7.6) contained 28 mM sodium HEPES, 50 mMKOAc, 1 mM$Mg(OAc)_2$, 1 mM ATP, 28 μM GTP, 9 mM creatine phosphate, 40 μg/ml of creatinephosphokinase, 0.5 mM spermidine, 2 mM DTT and 28 μM each of an amino acids mixture minus methionine, 0.8 MBq of [^{35}S]-methionine (37 TBq/mmol, MUROMACHI YAKUHIN KAISHA LTD.), 2.5 μl of wheat-germ extract, 1 μg of bovine liver tRNA and 0.5 μl of transcribed mRNA. Incubations were carried out at 26°C for 90 min. Translated wild-type- or M457L- PS1–CTFs in SDS–PAGE sample buffer without heat treatment were run on 12.5% SDS–PAGE, and detected by fluorography or western blotting.

Antibodies—GST-mouse PS1-loop, that is a recombinant glutathione S-transferase (GST) fusion protein including mouse PS1 332–371 aa region, expressed in *E. coli* using pGEX 5×-1 (GE Healthcare), was injected into rabbits (*7*). MBP–human PS1-loop, that is a recombinant maltose-binding protein (MBP) fusion protein including the human PS1 263–407 aa region, expressed in *E. coli* using pMAL-c2 (New England BioLabs), was used as the ligand for purification of anti-PS1-loop IgG. Purified MBP-human PS1-loop was coupled with Affi-Gel 15 (Bio-Rad) for purification of anti-PS1-loop IgG. Affinity purified IgG, named anti-PS1-loop, reacts with the corresponding region of rat and human PS1–CTF.

Western Blotting—For the western blot analysis of PS1–CTF, samples dissolved in SDS–PAGE sample buffer without heat treatment were run on 12.5% SDS–PAGE gels, transferred to polyvinylidenedifluoride (PVDF) membranes (Bio-Rad), and probed with anti-PS1-loop antibody. The immunoblots were developed using an ECL system (GE Healthcare), and [^{35}S]-labelled PS1-CTF was detected on X-ray film or by LAS-3000 (Fuji Film co. Japan).

Preparation of cultured cell extract—HeLa, HEK293 and COS-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum and penicillin–streptomycin. Cells in 10-cm dishes were collected and re-suspended in cell extraction buffer (50 mM Tris, 0.1% SDS, 10 μM pepstatin, 10 μM chymostatin, 10 μM antipain, 10 μM leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, pH 7.5) and sonicated for 30 s.

Other Methods—Radioactivity of [^{35}S]-labelled PS1–CTFs on the diphenyloxazole-impregnated dried gels and PVDF membranes were quantitated by counting protein bands with a liquid scintillation counter (*23*). The counting efficiencies of the radioactivities on the diphenyloxazole-impregnated dried gels and PVDF membranes were 77% and 92%, respectively.

RESULTS

Characterization of In Vitro Translated PS1-CTF as a Standard Protein—Endogenous PS1 undergoes endoproteolysis, generating approximately a 30-kDa N-terminal fragment (NTF) and a 20-kDa CTF. It was reported that the cleavage sites of human PS1 overproduced in HEK293 cells are between ^{298}Met-^{299}Ala, ^{291}Thr-^{292}Met and ^{292}Met-^{293}Val, and the major PS1–CTF starts at ^{299}Ala and minor PS1–CTFs start at ^{292}Met or ^{293}Val (*12*). The N-terminal amino acid sequence of PS1–CTF purified from rat liver was also examined and it was found that almost all PS1–CTF start at ^{299}Ala (Chai, H.L. and Miura, S., unpublished data). To investigate the amount of PS1–CTF in various rat tissues by western blot analysis, standard PS1–CTF protein must be necessary. Though an attempt was made to produce PS1–CTF in *E. coli* as a standard protein that has endogenous PS1–CTF sequence plus an initiator methionine at the N-terminus, a recombinant PS1–CTF protein could not be obtained probably because of its high hydrophobicity (*15–19*). Therefore, an *in vitro* translation system was chosen to produce PS1–CTF using wheat-germ extract. The translation mixture was run on SDS–PAGE, and translated PS1–CTF (TL-PS1–CTF) was detected by fluorography and western blotting using anti-PS1-loop antibody (Fig. 1). About 20-kDa polypeptide of TL-PS1–CTF was clearly detected on the fluorogram and western blotting (Fig. 1, lanes 2 and 4). The observed faint band nearly 40 kDa (Fig. 1, lane 4) was probably a dimer of TL-PS1–CTF. No polypeptides were observed in the translation mixture without PS1-CTF transcript (Fig. 1, lanes 1 and 3).

Transfer Efficiency of TL-PS1–CTF—To determine the precise amount of PS1–CTF in tissues using western blot analysis, transfer efficiency of TL-PS1–CTF as a standard protein to the PVDF membrane is essential. Various volumes of translation mixture were loaded on

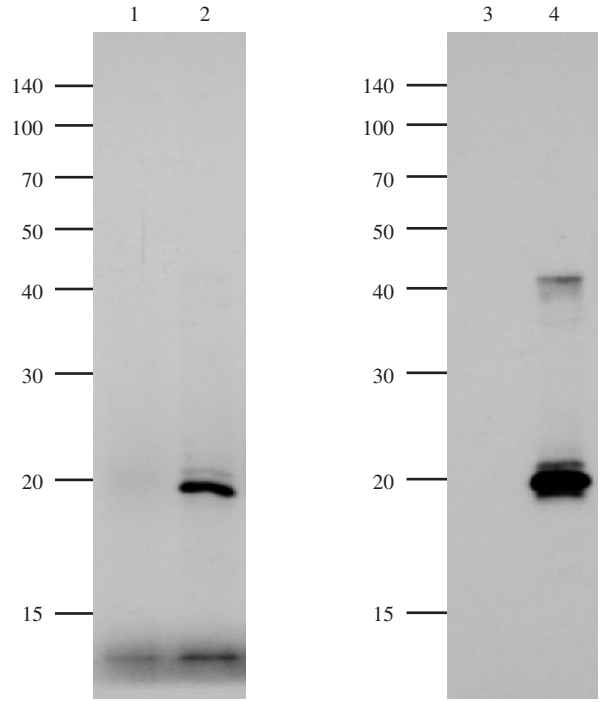

Fig. 1. ***In vitro* translation of PS1-CTF.** TL-PS1–CTF consisting of 299–467 aa of PS1 with initiatior Met at N-terminus, synthesized using a wheat-germ cell free protein synthesizing system (lanes 2 and 4), and negative control wheat-germ translation mixture without mRNA (lanes 1 and 3) were analysed on 12.5% SDS–PAGE followed by fluorography (left panel: 13-h exposure) or western blotting with anti-PS1-loop antibody (right panel: 2-min exposure). Molecular mass standards are shown on the left in kilodaltons.

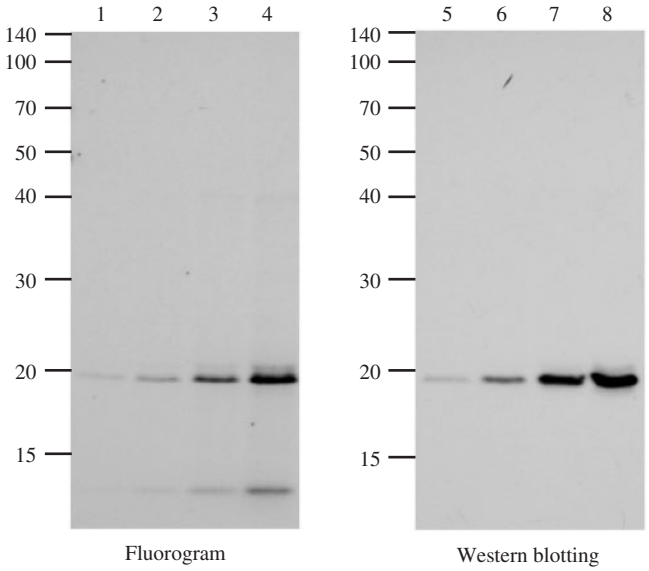

Fig. 2. **Determination of PS1-CTF transfer efficiency to the PVDF membrane.** Various volumes of translation mixture (31.25 nl: lanes 1 and 5, 62.5 nl: lanes 2 and 6, 125 nl: lanes 3 and 7, 250 nl: lanes 4 and 8) were analysed by 12.5% SDS–PAGE and PS1–CTF was detected by fluorography (left panel: 70-h exposure) or western blotting using anti-PS1–CTF antibody (right panel: 2-min exposure). Molecular mass standards are shown on the left in kilodaltons.

Table 1. **Transfer efficiency of TL-PS1-CTF.**

Translation mixture (nl)	Fluorogram (dpm)	Western blot (dpm)	Transfer efficiency (%)	Mean of transfer efficiency (%)
31.25	106	90	85	
62.5	203	160	79	80.5
125	435	318	73	
250	827	702	85	
1,000	3,357[a]			

[a]Deduced mean value from four experimental data.

two sets of SDS–PAGE, and TL-PS1–CTF was determined by fluorography (Fig. 2 lanes 1–4) and western blot analysis using anti-PS1-loop antibody (Fig. 2 lanes 5–8). The bands corresponding to TL-PS1–CTF on the fluorogram and western blot were excised and radioactivities of those bands were quantified by liquid scintillation counter (Table 1). Transfer efficiency of TL-PS1–CTF to PVDF membrane in the experimental condition of this study was from 75–85% and mean value was ~80%; the deduced radioactivity in the translation mixture was 3,357 dpm/μl.

Amount of PS1–CTF in Various Tissues—To investigate the amount of PS1–CTF in various tissues, four kinds of tissue homogenate from rat were prepared: liver, kidney, heart and brain. Protein concentration of each homogenate was determined by Lowry's method (*22*), and their protein profiles are shown in Fig. 3. It was obvious that the amount of total protein in each homogenate was almost similar, but expressed protein species in homogenates were quite different. After the appropriate amount of PS1–CTF in four tissue homogenates were investigated by SDS–PAGE and western blotting analysis (data not shown), four different volumes of translation mixture including various amount of TL-PS1–CTF and 20 μg, 10 μg, 20 μg and 80 μg protein of liver, kidney, brain and heart homogenates, respectively, were applied to SDS–PAGE and analysed by western blotting (Fig. 4A). It was clear that the intensity of TL-PS1–CTF was linearly increased (Fig. 4, lanes 1–4), and the intensity of PS1–CTF in the four tissue homogenates (Fig. 4, lanes 5–8) were between those of TL-PS1–CTF in lane 1 and lane 4. The intensity of TL-PS1–CTF bands was quantified (by LAS-3000 mini) and was considered as the standard curve for the determination of PS1–CTF in the homogenate (Fig. 4B). The PS1–CTFs in liver, kidney, brain and heart were corresponding to 87.5, 112.5, 84.4 and 39.1 nl of translation mixture, respectively.

Calculation of PS1–CTF in Tissue—TL-PS1–CTF theoretically has 457th Met and an additional initiator Met at N-terminus (Fig. 5A, upper panel). It was reported that initiator Met was removed when its preceded residues with a side chain having a radius of gyration of 1.29 Å or less: Val, Gly, Ala, Ser, Cys and Thr (*24*).

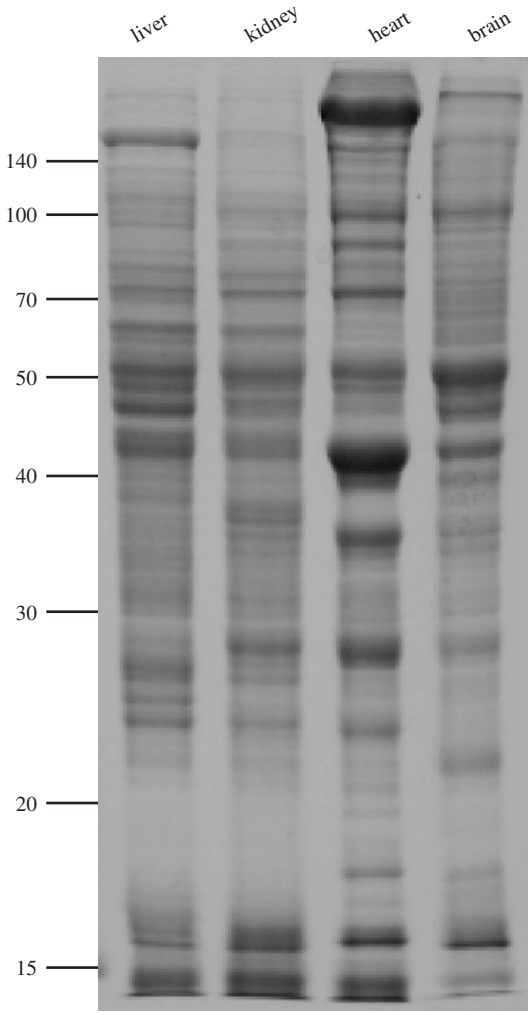

Fig. 3. **Protein profiles of various tissues homogenates from rat.** Same amount of tissue homogenates from rat (liver, kidney, brain and heart: 20 μg of each protein) were analysed by 12.5% SDS–PAGE, and protein bands were visualized with CBB. Molecular mass standards are shown on the left in kilidaltons.

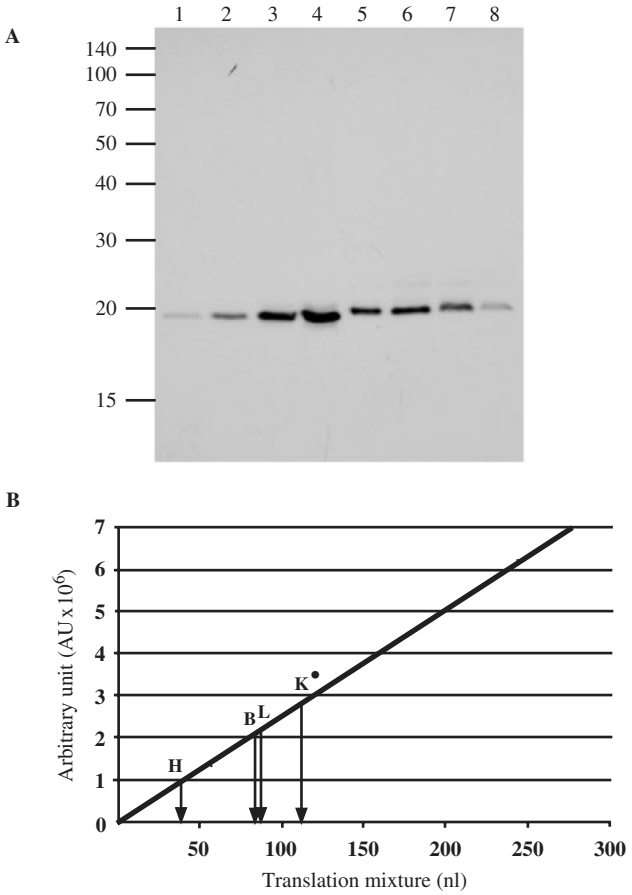

Fig. 4. **PS1–CTF content in various tissues homogenates from rat.** (A) Various amounts of TL-PS1–CTF and tissue homogenates from rat were analysed by 12.5% SDS–PAGE and PS1–CTF detected by western blotting. Lanes 1–4 correspond to 31.25 nl (lane 1), 62.5 nl (lane 2), 125 nl (lane 3) and 250 nl (lane 4) of translation mixture, respectively. Lanes 5–8 correspond to liver (20 μg protein: lane 5), kidney (10 μg protein: lane 6), brain (20 μg protein: lane 7) and heart (80 μg protein: lane 8) homogenates, respectively. Molecular mass standards are shown on the left in kilodaltons. (B) Standard curve for determination of PS1–CTF was made by quantification of PS1–CTF on the PVDF membrane (Fig. 4A: lanes1–4) using LAS-3000 mini (Fuji Film co. Japan). L, liver; K, kidney; B, brain; H, heart.

As the preceded residue is Ala in the case of TL-PS1–CTF, initiator Met of TL-PS1–CTF is predicted to be removed. To confirm whether the initiator Met of TL-PS1–CTF was removed or not, ^{457}Met was changed to Leu (Fig. 5A, lower panel, TL-PS1–CTF M457L). TL-PS1–CTF M457L synthesized *in vitro* was analysed by fluorography and western blotting (Fig. 5B). Though TL-PS1–CTF and TL-PS1–CTF M457L were detected by western blot analysis (Fig. 5B, lanes 5 and 6), TL-PS1–CTF M457L was not detected by fluorography (Fig. 5B: lane 3). This result clearly showed that the initiator Mets of TL-PS1–CTF and TL-PS1–CTF M457L were removed and TL-PS1–CTF contains only one Met residue. Taking into consideration nearly 1 μl of translation mixture contains 3,357 dpm of TL-PS1–CTF (Table 1), specific radioactivity of [^{35}S]-Met is 2,220 dpm/fmol, the amount of PS1–CTFs in liver, kidney, brain and heart were corresponding to 87.5 (per 20 μg protein), 112.5 (per 10 μg protein), 84.4 (per 20 μg protein) and 39.1 nl (per 80 μg protein) of translation mixture, respectively, and TL-PS1–CTF has only one Met residue, accurate amount of PS1–CTF in tissue homogenates were calculated and presented in Table 2. Each PS1–CTF content in liver, kidney, brain and heart were 6.6, 17.0, 6.4 and 0.7 fmol/mg protein, respectively. PS1–CTF content in kidney was almost 2.5-fold higher than those in liver and brain, and PS1–CTF content in heart was almost 1/10 of that in liver.

Determination of PS1–CTF in Various Cultured Cell Lines—Four different amounts of kidney homogenate as a standard of PS1–CTF and 11 μg, 8 μg and 14.5 μg protein of HeLa, HEK293 and COS-1 cell extract, respectively, were applied to SDS–PAGE and analysed by western blotting (Fig. 6). A standard curve with four

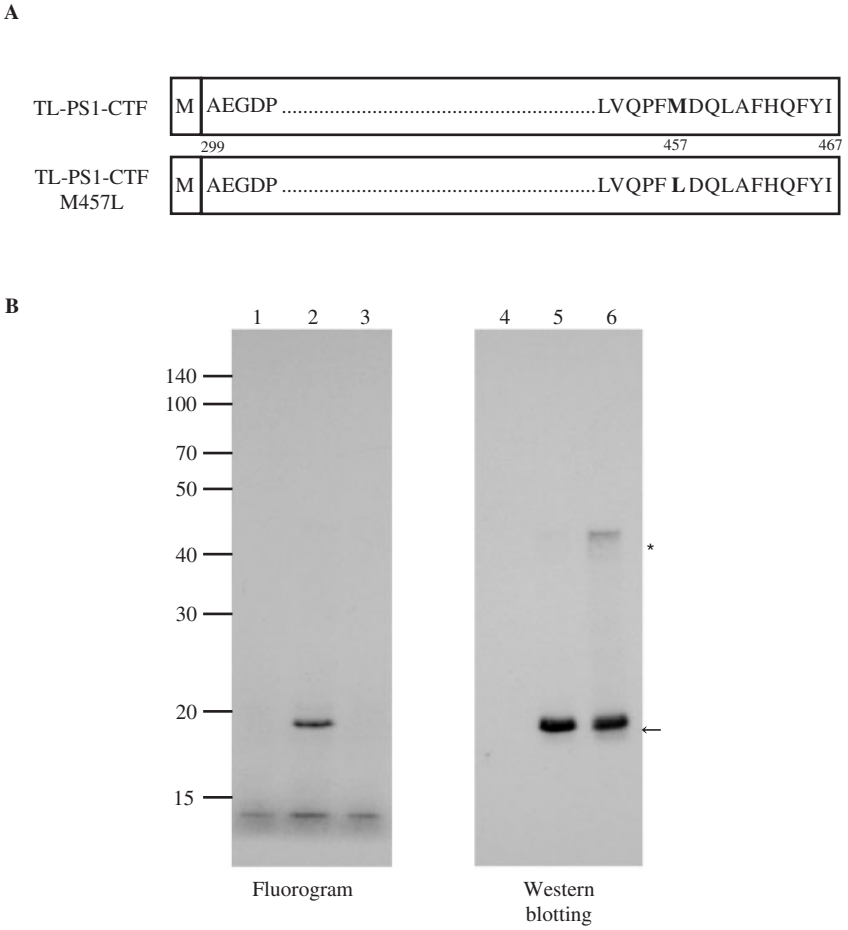

Fig. 5. **Absence of initiation methionine residue of PS1–CTF synthesized *in vitro*.** (A) Schematic representation of *in vitro* translated PS1–CTFs. TL-PS1–CTF consists of C-terminal 169 residues of human PS1 (299–467 aa of PS1) and an initiator Met at the N-terminus. TL-PS1–CTF M457L is similar to TL-PS1–CTF but 457th Met is substituted with Leu. (B) TL-PS1–CTF (lanes 2 and 5), TL-PS1–CTF M457L (lanes 3 and 6) and negative control wheat-germ translation mixture without mRNA (lanes 1 and 4) were analysed on 12.5% SDS–PAGE followed by fluorography (left panel: 13-h exposure) or western blotting with anti-PS1-loop antibody (right panel: 2-min exposure). Molecular mass standards are shown on the left in kilodaltons. Asterisk probably shows a dimer of TL-PS1–CTF M457L.

Table 2. **PS1-CTF content in various tissues from rat.**

Tissue	Weight (g)[a]	Total protein (mg)	PS1–CTF content		Total PS1–CTF (pmol)
			fmol/mg protein	pg/mg protein[b]	
Liver	21.44	4180	6.6	122.9	27.6
Kidney	4.47	782	17.0	316.6	13.3
Brain	3.37	348	6.4	119.2	2.2
Heart	2.46	395	0.7	13.0	0.3

[a]Tissue weights were obtained from two rats. [b]Calculated from deduced molecular weight of rat PS1–CTF (18,624 Da).

different amounts of kidney extracts were made as indicated in Fig. 4B and the amount of PS1–CTF was determined (Table 3). PS1–CTF content in COS-1 cell was almost 2.7-fold higher than those in HeLa and HEK293 cells.

DISCUSSION

PS1 mRNA has been shown to be expressed in all mammalian tissues (*2*, *6*, *21*). On the other hand, PS1 protein expression has been qualitatively investigated by western blotting analysis (*12*, *21*). Recently, Sato T. *et al*. (*20*) showed that active γ-secretase complex contain only one component each of PS1, Pen2, nicastrin and Aph1. Though they showed that active γ-secretase complex contained equivalent molecules of four components by semi-quantitative western blotting analysis using various anti-tag antibodies, there has been no report on the accurate amount and content of endogenous PS1 in mammalian tissues or cultured cells. This study initially showed the accurate amount and content of endogenous PS1 in liver, kidney, brain and heart rat tissues or conventionally used cultured cells such as HeLa, HEK293 and COS-1. In rat tissues, the contents of PS1–CTF per milligram protein were in the order kidney > liver = brain > heart. Incidentally, the contents of PS1–CTF in liver and brain were 6.6 and 6.4 fmol/mg protein respectively,

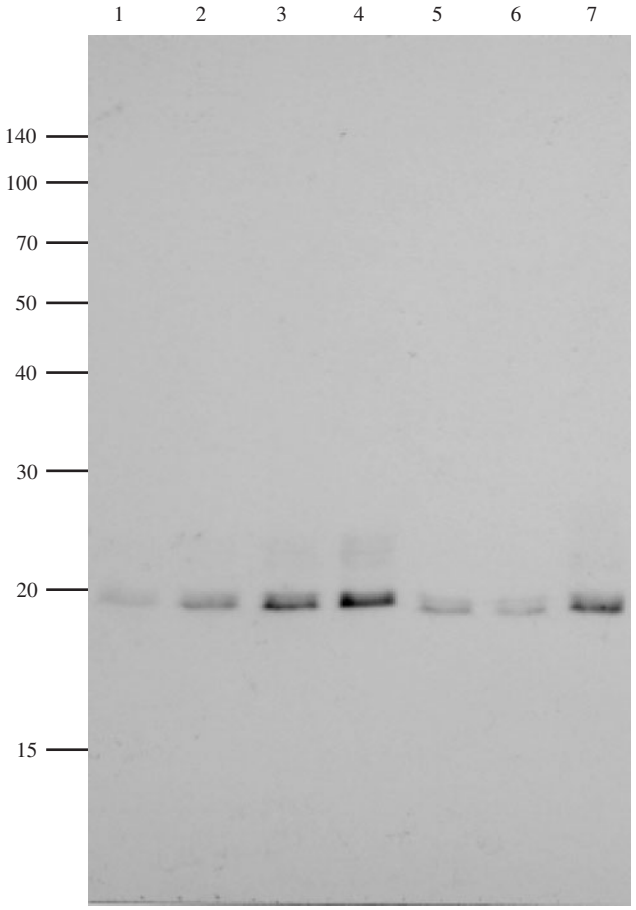

Fig. 6. **PS1–CTF content in various cell lines.** Various amounts of kidney extract and homogenates from various cell lines were analysed by 12.5% SDS–PAGE and PS1–CTF detected by western blotting. Lanes 1–4 correspond to 1.25 μg (lane 1), 2.5 μg (lane 2), 5 μg (lane 3) and 10 μg (lane 4) of kidney extract, respectively. Lanes 5–7 correspond to HeLa (8.25 μg protein: lane 5), HEK293 (6 μg protein: lane 6) and COS-1 (7.25 μg protein: lane 7) extract, respectively. Molecular mass standards are shown on the left in kilodaltons.

Table 3. **PS1-CTF content in cultured cell lines.**

	Count of cells	Extract (μl)	Protein (mg)	CTF (fmol)	PS1–CTF content	
					fmol/mg protein	fmol/10^5 cells
HeLa	2×10^7	400	8800	83.4	9.48	0.417
HEK293	1.3×10^7	364	5824	57.8	9.92	0.445
COS1	2.5×10^6	50	1450	38.1	26.26	1.050

the content of PS1–CTF in kidney is 2.6-fold higher than in liver, and the contents of PS1–CTF in heart was almost 1/10 of liver. As PS1–CTF is one of the components of γ-secretase, this result imagines that γ-secretase activity in kidney may be higher than that in another tissues, and expression level of substrates of γ-secretase such as Notch, ErbB4 and CD44 (*5*, *25–27*) may be higher in kidney than another tissues. γ-Secretase is involved in production of Aβ but also many other physiological phenomena through the cleavage of various substrates. Once abnormalities are seen in these phenomena, the investigation about the amount of each component of γ-secretase as well as the investigation of γ-secretase activity itself must be essential. Though several assay of γ-secretase *in vitro* have been already established (*28*, *29*), method for determination of the amount of γ-secretase components have never been established. This report is the first step for determination of the amount of γ-secretase components. The authors of this study are attempting to make good antibodies against other γ-secretase components in order to determine those amounts in tissues.

In cultured cell lines, the contents of PS1–CTF were determined by western blotting analysis using rat kidney homogenate as a standard protein instead of PS1–CTF synthesized *in vitro*. The contents of PS1–CTF per milligram protein were in the order COS-1 > HeLa = HEK293, the content of PS1–CTF in COS-1was 26.6 fmol/mg protein, and those in HeLa and HEK293 cells were almost one-third. As the COS-1 cell line is derived from kidney cells of the African green monkey, the content of PS1–CTF was higher than other cell lines. This result reflected that kidney had the highest content of PS1–CTF in the tissues investigated in this experiment. Although we expected that the content of PS1–CTF in HEK293 cells was nearly same as that in COS-1 cells, the content of PS1–CTF in HEK293 cells was one-third in COS-1 cells and almost similar to that in HeLa cells derived from cervical cancer cells. This may be due to the origin of HEK293 cells. HEK293 is not derived from adult kidney cells but embryonic kidney cells. This suggests that expression of PS1–CTF in embryonic kidney cells may be lower than that in adult kidney cells.

It is generally difficult to quantitatively determine the amount of specific protein in mammalian tissues. Especially in case of PS1, there are two obstacles. One is that expressed PS1 protein has been speculated to be extremely limited. Another is that PS1 is very hydrophobic and multiple membrane-spanning protein (*6–11*). The experiments conducted for this study could overcome the first obstacle to get a good antibody named anti-PS1-loop that recognized 50 attomoles of PS1–CTF on western blotting membrane (Fig. 4A, lane 1). By the second obstacle, we were not able to produce the PS1–CTF in *E. coli* that was used as a standard protein for the determination of PS1–CTF in mammalian tissues and cultured cell lines. However, it was possible to use *in vitro* protein synthesis system using wheat-germ extract in place of the *E. coli*. protein synthesizing system. It is to be noted that the data reported in this study are based on the calculation that 1 μl of translation mixture contains 3,357 dpm of [^{35}S]-Met corresponding to 1.512 fmol of PS1–CTF. This is based on the assumption that the wheat-germ extract, used for the synthesis of PS1-CTF, includes no endogenous free Met. This assumption is probably correct because free amino acids were removed by gel chromatography procedure for the preparation of wheat-germ extract. Here, our result reported here makes it possible to investigate the change of PS1–CTF content in the brain with ageing. This may clue us to understand the pathology of sporadic AD. In the field of biochemistry, the method reported here must be

applicable to another limited amounts of membrane proteins in the tissues such as receptor proteins, pore forming proteins and translocator proteins.

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CONFLICT OF INTEREST

None declared.

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