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Appearance of Sodium Dodecyl Sulfate-Stable Amyloid β-Protein (Aβ) Dimer in the Cortex During Aging

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We previously noted that some aged human cortical specimens containing very low or negligible levels of amyloid β-protein (Aβ) by enzyme immunoassay (EIA) provided prominent signals at 6~8 kd on the Western blot, probably representing sodium dodecyl sulfate (SDS)-stable Aβ dimer. Re-examination of the specificity of the EIA revealed that BAN50- and BNT77-based EIA, most commonly used for the quantitation of Aβ, capture SDS-dissociable Aβ but not SDS-stable Aβ dimer. Thus, all cortical specimens in which the levels of Aβ were below the detection limits of EIA were subjected to Western blot analysis. A fraction of such specimens contained SDS-stable dimer at 6~8 kd, but not SDS-dissociable Aβ monomer at ~4 kd, as judged from the blot. This Aβ dimer is unlikely to be generated after death, because (i) specimens with very short postmortem delay contained the Aβ dimer, and (ii) until 12 hours postmortem, such SDS-stable Aβ dimer is detected only faintly in PDAPP transgenic mice. The presence of Aβ dimer in the cortex may characterize the accumulation of Aβ in the human brain, which takes much longer than that in PDAPP transgenic mice. ***(Am J Pathol 1999, 154:271–279)***

One of the great strides made in recent research on Alzheimer's disease (AD) is the generation of transgenic mice exhibiting AD-like pathology with innumerable diffuse and neuritic plaques throughout the cortex.[1–3] In PDAPP transgenic mice overexpressing β-amyloid precursor protein (APP) V717F, the levels of amyloid β-protein (Aβ) 42, a longer species of Aβ, dramatically increase in the hippocampus and cortex at 4 months of age and mature plaques appear at 8 months of age.[1,4] The structural alterations surrounding mature plaques are very similar to those found in AD brains; degenerating neuronal processes, reactive astrocytes, and activated microglia are seen in these lesions.[5,6] However, there is a significant difference in Aβ accumulation between humans and the transgenic mice. In the transgenic mouse brain, it takes only ~14 months for Aβ accumulation, which starts at 4 months, to reach the levels seen in the occipitotemporal cortex of human brain.[4] In humans, it presumably takes 20 years or more to reach similar levels of Aβ42 in the cortex.[7]

Aβ42, although a minor Aβ species, has received particular attention because (i) it has a higher aggregation potential than Aβ40, a major secreted species,[8] (ii) immunocytochemistry and two-site enzyme immunoassay (EIA) have revealed that Aβ42 is the initially deposited species in the brain,[9,10] and (iii) all APP mutations, and presenilin 1 and 2 mutations linked with familial AD (FAD), accompany increased secretion of Aβ42.[11–13] In fact, plasma from FAD pedigrees[14,15] and Down syndrome patients,[16] who invariably develop AD pathology in middle age, contains significantly higher levels of Aβ42. In addition, the proportion of Aβ42 in the Aβ deposited in FAD brains is significantly higher than that in sporadic AD brains.[17] Thus, several lines of transgenic mice incorporating mutant APP and/or presenilin genes may be excellent models of FAD.[1–3]

However, sporadic AD, which is far more prevalent than FAD and is believed to be a polygenic disease, is not associated with increased levels of Aβ42 in plasma.[14] It is of note that the ApoE4 allele (ε4), a strong risk factor for AD, is associated with neither an increased number of Aβ42-positive plaques nor increased deposition of Aβ42 in the brain.[18,19] Nevertheless, sporadic AD patients and a substantial proportion of elderly people exhibit exten-

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sive deposition of Aβ42 in the brain.[7,20] Thus, it is reasonable to speculate that some unidentified factors other than increased secretion of Aβ42 are involved in Aβ deposition in sporadic AD patients and among the general aged population. Consequently, it is of particular importance to investigate autopsied human brains despite potentially confounding postmortem artifacts.

We previously quantitated the Aβ levels in the cortex and subcortical regions during aging.[7,20] There was a strong tendency toward Aβ42 accumulation between the ages of 50 and 70 years in T4, putamen, and mamillary body, and a little later in CA1.[7,20] Even in cases in which no senile plaques were immunocytochemically detected, EIA clearly showed that significant amounts of Aβ42 had already accumulated.[7] In contrast to Aβ42, Aβ40 showed no apparent age-dependent accumulation, and high levels of Aβ40 were found to be associated with AD.[7] In the course of this work, we noted that Aβ dimer at 6~8 kd, but not Aβ monomer at ~4 kd, is often prominent on the Western blot of specimens showing negligible levels of Aβ42 by EIA.[20] Further investigation has clarified that (i) BAN50- or BNT77-based EIA quantitates sodium dodecyl sulfate (SDS)-dissociable Aβ at ~4 kd, but not SDS-stable Aβ dimer at 6~8 kd, and (ii) specimens containing negligible amounts of Aβ as determined by EIA often contain detectable levels of SDS-stable Aβ dimer on the Western blot. Although we currently do not know the exact significance of the Aβ dimer, it is possible that the SDS-stable Aβ dimer accumulates very slowly and plays an important role in the initial stages of β-amyloidogenesis in human brain.

Materials and Methods

Subjects

The present study is based on autopsies performed (n = 74; 56 men, 18 women) during the period 1995–97 at the Tokyo Medical Examiner's Office (Otsuka, Tokyo), as described previously.[7,20] The ages at death of the 74 subjects ranged from 24 to 92 years (3 at 20–29 years, 4 at 30–39 years, 17 at 40–49 years, 18 at 50–59 years, 13 at 60–69 years, 10 at 70–79 years, 8 at 80–89 years, and 1 at 92 years). Postmortem delay ranged from 2 to 24 hours. The other source of autopsy cases (n = 40; 28 men, 12 women) was the Gunma Cancer Center (Ohta, Gunma); all of these cases had malignant neoplasms. Their ages at death ranged from 40 to 81 years (5 at 40–49 years, 12 at 50–59 years, 9 at 60–69 years, 13 at 70–79 years, and 1 at 81 years) and postmortem delay ranged from 1 to 13 hours (see Table 2).

Tissue Preparation

Cortical pieces of CA1 and T4 at the level of lateral geniculate body, approximately 80–110 mg each, were sampled from fresh brains at autopsy at the Tokyo Medical Examiner's Office and stored at −80°C until use. The attached leptomeninges and vessels were carefully dissected out. At the Gunma Cancer Center, cortical blocks were obtained from the prefrontal cortex (Brodmann 9, 10, and 11) and stored at −80°C until use. Pieces weighing approximately 200 mg were processed for EIA and Western blotting.

PDAPP transgenic mice, aged 9.3–9.7 months,[1,4] were used to examine the effects of postmortem delay on the molecular form of Aβ. After death, two each of 12 mice were kept at room temperature for 0, 2, 4, 6, 12, or 18 hours, then frozen at −80°C until use. The mouse brains were similarly processed for EIA and Western blotting.

Tissue Extraction

Each of the sampled pieces was homogenized with a Dounce homogenizer (20 strokes) in 4 volumes of Tris-saline (50 mmol/L Tris-HCl, pH 7.6, 0.15 mol/L NaCl) containing 1 mmol/L EGTA, 0.5 mmol/L diisopropyl fluorophosphate, 0.5 mmol/L phenylmethylsulfonyl fluoride, 1 mg/L N^{α}-p-tosyl-L-lysine chloromethyl ketone, 1 mg/L antipain, 0.1 mg/L pepstatin, and 1 mg/L leupeptin. Each homogenate was further homogenized with a motor-driven Teflon/glass homogenizer (20 strokes) and centrifuged at 265,000 × g for 15 minutes on a TL 100.3 rotor in a TLX centrifuge (Beckman, Palo Alto, CA). For the cortical blocks from the Gunma Cancer Center, the Dounce homogenization step was omitted. The resultant pellet, after being washed once, was further extracted with more than 100 volumes (with respect to the initial tissue volume) of 70% formic acid. The homogenate was centrifuged on a TL 100.3 rotor as mentioned above. The supernatant was neutralized with NaOH and trizma base and subjected to the EIA.

Enzyme Immunoassay

The two-site EIA for Aβ consisted of a combination of five monoclonal antibodies: BAN50, BNT77, 4G8, BA27, and BC05. BAN50, BNT77, or 4G8 (Senetek PLC, St. Louis, MO; the epitope is located in Aβ17–24) was coated as a capture antibody on a multiwell plate (Immunoplate I, Nunc, Roskilde, Denmark). BAN50 (the epitope is located in Aβ1–10) presumably captures full-length Aβ, whereas BNT77 (the epitope is thought to be located in Aβ11–16)[21] is considered to capture all Aβ species truncated up to position 10, but not p3 which starts at Aβ17. Either BA27 specific for Aβ40 or BC05 specific for Aβ42 was used as a detection antibody following conjugation with horseradish peroxidase.

Aliquots (100 μl) of appropriately diluted formic acid extracts, as well as a synthetic peptide, Aβ1–40 or Aβ1–42 (Bachem, Torrance, CA), dissolved in dimethylsulfonyloxide, were applied to a BAN50-, BNT77-, or 4G8-coated multiwell plate and the loaded plate was incubated at 4°C overnight. After being rinsed with phosphate-buffered saline, the loaded wells were incubated with horseradish peroxidase-conjugated BA27 or BC05 at room temperature for 6 hours. Bound enzyme activity was measured using the TMB Microwell Peroxidase Sub-

strate System (Kirkegaard & Perry Labs, Gaithersburg, MD). For the insoluble Aβ42, the detection limit of EIA was 12 pmol/g wet weight.[22]

Western Blotting

Small aliquots (10 μl) of the formic acid extracts of the insoluble fractions were dried by Speed Vac (Savant Instruments, Farmingdale, NY), and solubilized with sample buffer (50 mmol/L Tris-HCl (pH 6.8), 12% glycerol, 2% SDS, 2.5% mercaptoethanol, 4 mol/L urea). These samples were subjected to Tris/tricine gel electrophoresis and the separated proteins were blotted onto a nitrocellulose membrane (pore size 0.22 μm, Schleicher & Schuell, Dassel, Germany). The blot, after heat treatment,[23] was incubated with BAN50, BA27, BC05, or BC65 (specific for Aβ43).[24] After washing with Tris-saline-based buffer, the blot was further incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Transduction Laboratories, Lexington, KY). Bound antibodies were visualized using the enhanced chemiluminescence system (Amersham, Buckingham, UK). This modified version of Western blotting[23] detected as little as 10 pg (2.5 fmol) of Aβ1–42 or Aβ1–40 per lane.

Besides specimens, synthetic Aβ1–40 or 1–42 (10, 20, 50, and 100 pg) was loaded onto each gel for Western blot quantitation of Aβ. SDS-stable Aβ dimer was quantitated using a standard curve for SDS-dissociable Aβ (synthetic Aβ) and the concentration was expressed as the Aβ monomer equivalent. Thus it was postulated that the blotting efficiency and BA27 or BC05 reactivity of SDS-stable dimer are the same as those of SDS-dissociable Aβ. Quantitation of enhanced chemiluminescence bands of interest was performed with a model GS-700 imaging densitometer on Molecular Analyst software (Bio-Rad Laboratories, Hercules, CA).

Aβ Immunocytochemistry

The formalin-fixed cortical blocks from the Tokyo Medical Examiner's Office were dehydrated and embedded in paraffin in a routine manner and cut into 6-μm-thick sections. Sections were immunostained with 4G8 (Senetek PLC; specific for Aβ17–24) by the avidin-biotin method (Vectastain Elite, Vector Laboratories, Burlingame, CA), after formic acid treatment.[7]

The cortical blocks from the Gunma Cancer Center were sliced to ~5 mm in thickness and fixed in 4% paraformaldehyde or 10% formalin in phosphate buffer for 24–48 hours at 4°C. Sections, 6 μm thick, were similarly immunostained with Aβ polyclonal antibodies.[25]

Apolipoprotein E Genotyping

Typing of the apolipoprotein E genotype was performed using the polymerase chain reaction (PCR) as described previously.[26]

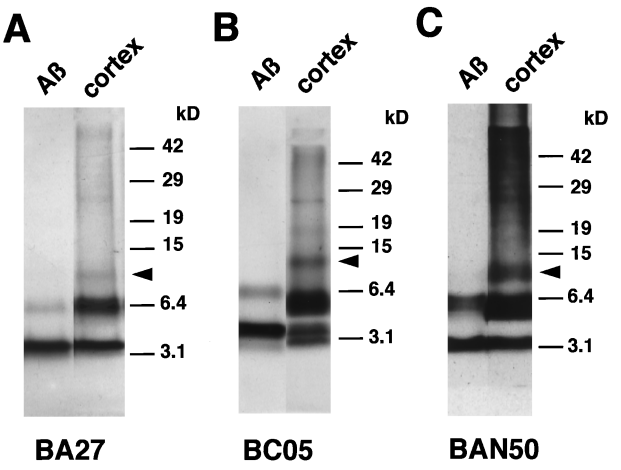

Figure 1. Western blots of the insoluble fraction from an aged occipital cortex. The formic acid extract, together with 100 pg of synthetic Aβ1–40 (A, left lane) or Aβ1–42 (B, left lane), or 5 ng of Aβ1–42 (C, left lane), was subjected to SDS polyacrylamide gel electrophoresis and Western blotting with BA27 (A), BC05 (B), or BAN50 C. In these three panels, Aβ40 or Aβ42 monomer is migrated at~4 kd, while Aβ40 or Aβ42 dimer to ~6 kd, and trimer to ~12 kd (arrowheads, A-C). In the right lane in B, the lowest band at ~3 kd presumably represents p3, namely, Aβ17–42, as judged by comparison of its mobility with that of synthetic Aβ17–42 (data not shown). The broader, somewhat fast-migrating, band in the right lane in B may reflect various amino-terminal truncations or modifications of Aβ42 (see Figure 2).

Results

BAN50- or BNT77-based EIA Quantitates a Dissociable Molecular Form of Aβ40 and Aβ42

When Aβ is extracted with formic acid from the insoluble fraction of aged or AD brains, three major molecular forms of Aβ40 or Aβ42 were observed on the Western blot: Aβ monomer at ~4 kd, dimer at ~6–8 kd, and larger oligomers and a smear (Figure 1). The latter two cannot be dissociated into 4-kd monomer with SDS or other harsh denaturants including guanidine hydrochloride (see Figure 2). Although these two Aβ species are not yet fully characterized, our data suggest their interrelationship: when Aβ monomer is present on the Western blot of a given brain homogenate, Aβ dimer can be also detected. In the insoluble fraction of human brain homogenate, the amount of SDS-stable Aβ dimer usually exceeds that of the dissociable Aβ form (Figure 1).

We have examined whether the EIA used quantitates the SDS-dissociable form of Aβ, the SDS-stable form, or both. β-Amyloid cores were partially purified from AD brain according to a previously reported protocol[27] and formic acid-extracted Aβ was fractionated in guanidine hydrochloride on a Superdex 75 column (Pharmacia, Uppsala, Sweden; HR10/30). By this procedure, dissociable Aβ monomer, SDS-stable Aβ dimer, and larger oligomers were readily separated according to their molecular sizes (Figure 2). BAN50- or BNT77-based EIA was found to specifically capture dissociable Aβ40 and Aβ42 species, but not SDS-stable dimer or oligomer (Figure 2). Thus, the BNT77-based EIA values most likely represent the levels of SDS-dissociable Aβ species, but not of SDS-stable Aβ dimer or oligomer. In contrast, 4G8 captured both dissociable Aβ and SDS-stable Aβ dimer. This is consistent

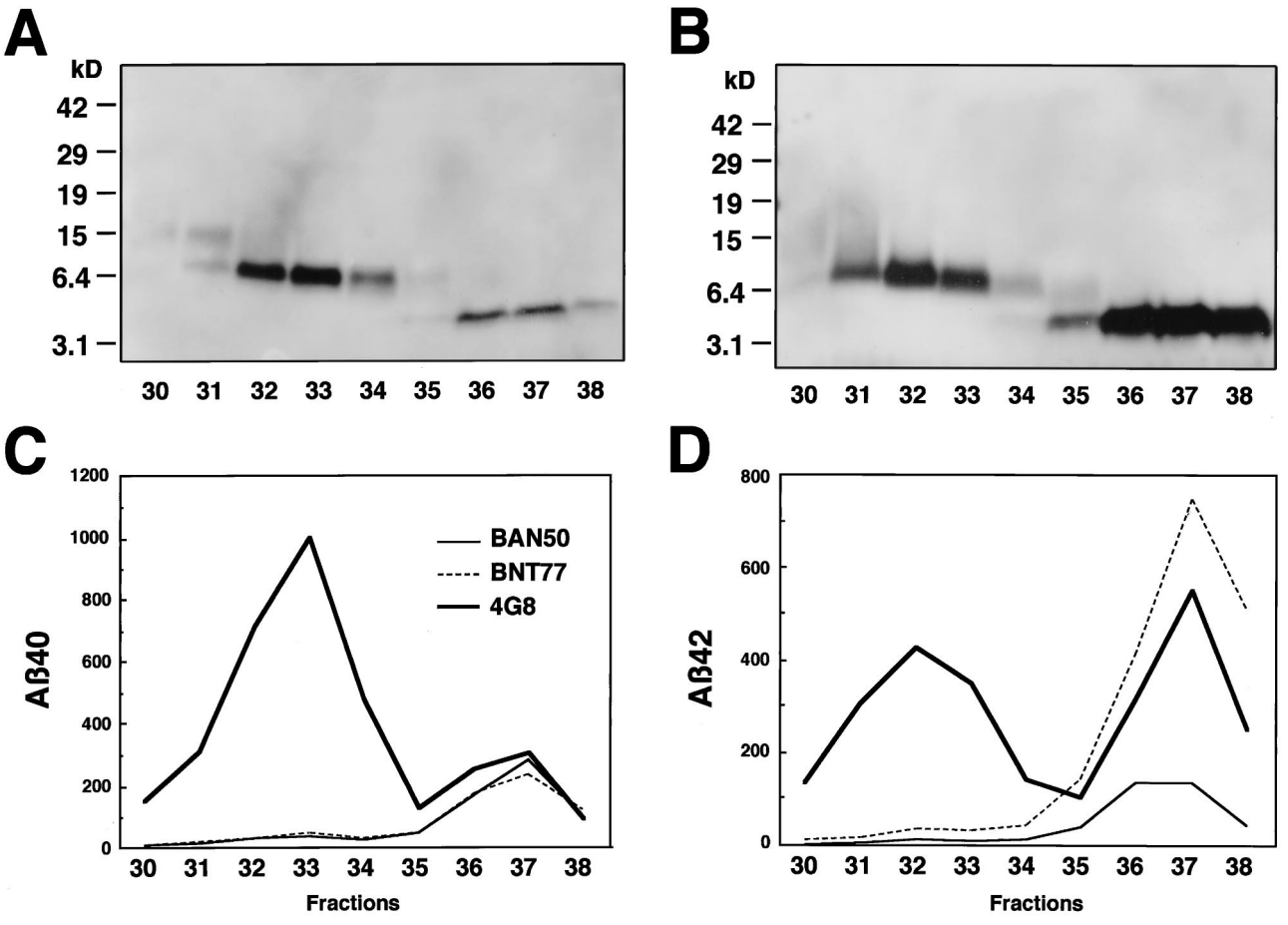

Figure 2. Western blot analysis and EIA of size exclusion chromatography fractions. The amyloid core-enriched fraction (ie, the SDS-insoluble, formic acid-soluble fraction) was prepared from AD brains according to a previously reported protocol.[27] The formic acid extract was dialyzed against 6 mol/L guanidine hydrochloride in 10 mmol/L phosphate buffer (pH 6.0), and the dialyzate was applied on a Superdex 75 HR10/30 (1.0 × 30 cm, Pharmacia) column which was preequilibrated and developed with 6 mol/L guanidine hydrochloride in 10 mmol/L phosphate buffer (pH 6.0) at a flow rate of 0.3 ml/min. Those fractions eluted at positions at 12 to 2.5 kd were subjected to EIA and Western blotting. For EIA an aliquot from each fraction was diluted with 10 volumes of buffer EC[22] and applied onto a BAN50-, BNT77-, or 4G8-coated plate. Bound Aβ was detected with peroxidase-labeled BA27 (**C**) or BC05 (**D**). For Western blotting each remaining fraction was dialyzed against 8 mol/L urea and the dialyzate was subjected to Tris/tricine SDS polyacrylamide gel electrophoresis. Each blot was probed with BA27 (**A**) or BC05 (**B**). BAN50-, BNT77-, or 4G8-based EIA values are indicated by a solid line, broken line, or bold line, respectively. Large differences between BNT77-based and BAN50-based Aβ42 values probably indicate great extents of truncations and modifications of Aβ42 (**D**).

with our previous observation that 4G8 preferentially labeled SDS-stable dimer on the blot (Shinkai Y, Morishima-Kawashima M, Ihara Y, unpublished observation).

SDS-Stable Aβ Dimer in Specimens in which Aβ is Undetectable by EIA

We examined whether specimens containing negligible levels (below the detection limit of 12 pmol/g wet weight) of Aβ42 by EIA contain SDS-stable Aβ dimer according to the sensitive Western blotting.[23] All these cases from two facilities showed no immunocytochemically detectable senile plaques in adjacent sections, as partly described before.[7] In a number of such specimens very prominent bands of Aβ dimer at 6~8 kd were seen (Figure 3, A and B), but Aβ monomer at ~4 kd was absent or scarcely detectable, an observation consistent with the above-described characteristics of the EIA. Because of the presence of a trace amount of the Aβ dimer in the sample (Table 1, A and B, and Table 2), we were unable to microsequence the molecule. However, we consider that the 6~8-kd band represents Aβ dimer because (i) BAN50, 4G8, and BC05 labeled the band on the blot (see below), (ii) truly end-specific BA27 (Morishima-Kawashima M and Ihara Y, unpublished observations) often labeled a band at the same position, (iii) occasionally, above the 6~8-kd band, a 12-kd band was observed, suggesting the presence of Aβ trimer (Figure 1, Figure 3, A and B), and thus strengthening the argument that the band at 6~8 kd represents Aβ dimer, and (iv) Western blots of many specimens showed that there is a transition from 6~8-kd band alone, to appearance of an additional band at ~4 kd, and finally to the mixture most commonly seen in aged brain in which the 6~8-kd band is considered to represent SDS-stable Aβ dimer (data not shown; Figure 1).

In several cases (93137 and 95303 in Table 1A, 94367 in Table 1B, and 21 and 40 in Table 2) in which Aβ was undetectable by EIA, Aβ42 monomer was detected on the blot in addition to the SDS-stable dimer. This may be partly due to the difference in sensitivity of the two assay methods: for quantitation of insoluble Aβ Western blotting

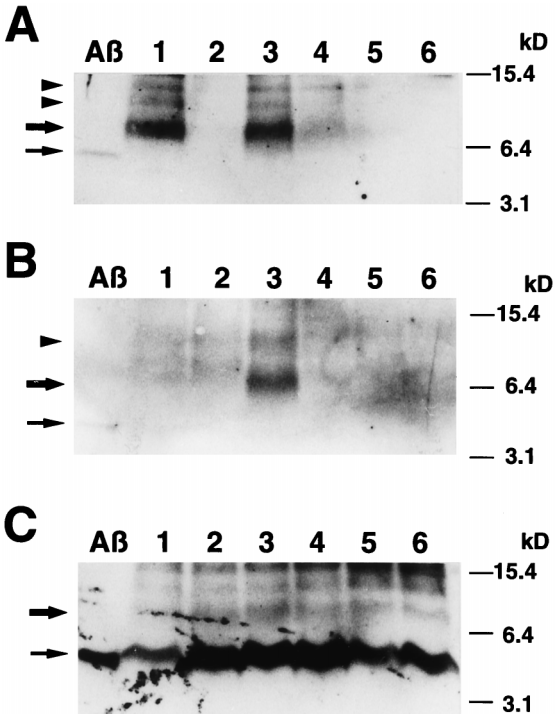

Figure 3. Western blots of representative EIA-negative specimens and PDAPP transgenic mouse brains. A, B: Representative cases showing the presence of SDS-stables Aβ42 (A) and Aβ40 (B) dimers. Each left-most lane is loaded 10 pg of synthetic Aβ1–42 (A) or Aβ1–40 (B). An upper arrowhead in A or an arrowhead in B indicate a 12-kd band, presumably representing Aβ trimer. A lower arrowhead in A indicates an ~8-kd band, perhaps representing anomalously folded Aβ42 dimer.[36] Small and large arrows in A and B indicate Aβ monomer and Aβ dimer, respectively. C: The effects of postmortem delay on the molecular form of Aβ42 in the transgenic mice. The mice were kept at room temperature for 0 (lane 1), 2 (lane 2), 4 (lane 3), 6 (lane 4), 12 (lane 5), and 18 (lane 6; see text) hours after death and processed for Western blotting with BC05. No immunoreactivity with BA27 was detected on the blot (data not shown). The leftmost lane is loaded 10 pg of synthetic Aβ1–42. Small and large arrows indicate Aβ42 monomer and Aβ42 dimer, respectively.

is more sensitive than EIA, which requires a neutralization step[22] that results in extensive dilution of the formic acid extract.

In T4, 26 cases showed negligible Aβ levels by EIA. Six cases had only Aβ40 dimer, 2 cases only Aβ42 dimer, and 5 cases had both; thus, there were 13 cases with Aβ40 or Aβ42 dimer (50%; Table 1A). CA1 shows somewhat different characteristics in the Aβ accumulation. As assessed by EIA quantitation, as compared to T4, Aβ accumulation starts a little later.[7] There were 32 cases in which Aβ in CA1 fell below the detection limit by EIA. Among them, 2 cases had only Aβ40 dimer, 7 cases only Aβ42 dimer, 6 cases had both; thus, 15 cases had Aβ40 or Aβ42 dimer (47%; Table 1B).

To exclude the possibility that the dimer is generated postmortem, cases from a local cancer hospital were similarly examined (Table 2). These cases were generally autopsied shortly after death (see Table 2). Even the specimens frozen very shortly after death contained Aβ dimer, strongly suggesting that the Aβ dimer is not an artifact generated postmortem. In 22 cases, insoluble Aβ42 was below the detection limit. One of these cases had only Aβ40 dimer, 8 cases had only Aβ42 dimer, and 6 cases had both; thus, there were 15 cases with Aβ dimer (68%; Table 2).

We also examined PDAPP transgenic mice aged 9.3–9.7 months at death, an age at which amyloid deposition is apparent (Table 3).[4] The transgenic mice were kept at room temperature up to 18 hours after death. Up to 12 hours, Aβ dimer was barely observed in the transgenic mice (Figure 3C). One of the mice kept for 18 hours was found to contain larger amounts of Aβ dimer than of Aβ monomer at 4 kd (data not shown). Most interestingly, in these transgenic mice, with the one exception mentioned above, only a trace amount of SDS-stable Aβ dimer was observed, whereas there was a prominent band at ~4 kd representing SDS-dissociable Aβ on the blot (Figure 3C). This result can also exclude the possibility that the SDS-stable dimers are generated during evaporation of formic acid, a step required for SDS polyacrylamide gel electrophoresis.

Appearance of SDS-Stable Aβ42 Dimer May Be Age-Dependent

We examined whether the appearance of SDS-stable dimer is age-dependent in the present two autopsy series. In T4, from the autopsy series at Tokyo Medical Examiner's Office, for ages at death < 50 years, SDS-stable Aβ40 and Aβ42 dimers were detected in 6 (46%) and 2 (15%) of 13 cases, respectively. For ages 50–59, SDS-stable Aβ40 and Aβ42 dimers were detected in 4 (44%) and 3 (33%) of 9 cases, respectively. For ages 60–69, SDS-stable Aβ40 and Aβ42 dimers were detected in 2 (67%) and 2 (67%) of 3 cases, respectively. The incidence of Aβ42 dimer, but not Aβ40 dimer, tended to increase with age but was not statistically significant (Mantel extension test, $P < 0.10$).

In CA1 from the same series, for ages at death < 50 years, SDS-stable Aβ40 and Aβ42 dimers were detected in 2 (15%) and 2 (15%) of 13 cases, respectively. For ages 50–59 years, SDS-stable Aβ40 and Aβ42 dimers were detected in 5 (42%) and 7 (58%) of 12 cases, respectively. For ages 60–69 years, SDS-stable Aβ40 and Aβ42 dimer were detected in 1 (17%) and 4 (67%) of 6 cases, respectively. This age-dependent increase in the incidence of Aβ42 dimer but not of Aβ40 dimer in CA1 was statistically significant (Mantel extension test, $P < 0.02$).

In the prefrontal cortex from the autopsy series at the Gunma Cancer Center, for ages at death < 50 years, SDS-stable Aβ40 and 42 dimer were detected in 0 (0%) and 1 (50%) of 2 cases, respectively. For ages 50–59 years, the SDS-stable Aβ40 and Aβ42 dimers were detected in 2 (40%) and 2 (40%) of 5 cases, respectively. For ages 60–69 years, SDS-stable Aβ40 and Aβ42 dimers were detected in 2 (33%) and 5 (83%) of 6 cases, respectively. For ages 70–79 years, SDS-stable Aβ40 and Aβ42 dimers were detected in 3 (50%) and 6 (100%) of 6 cases, respectively. This age-dependent increase in the incidence of the Aβ dimer in the prefrontal cortex was statistically significant (Mantel extension test, $P < 0.05$). Although the number of cases was small, the presence of Aβ dimer is presumably unrelated to ApoE genotypes (Table 1, A and B, and Table 2).

Table 1A. Western Blot Data on EIA-negative T4 Specimens from Autopsy Cases at Tokyo Medical Examiner's Office

Case	Age	Gender	ApoE	Aβ40(BA27)		Aβ42(BC05)	
				4kd	8kd	4kd	8kd
92726	67	M	3/3	−	−	−	± ↓
92818	61	M	2/3	−	−	−	+ ↑
93023	45	M	3/4	−	−	−	−
93026	57	F	3/3	−	−	−	−
93137	59	M	3/3	−	−	+ 1.9*	+ ↑
93334	48	M	3/3	−	−	−	−
93459	65	M	3/3	−	−	−	± ↓
93570	52	M	3/3	−	−	−	−
93650	44	M	3/3	−	2+ ↑	−	−
93666	44	M	3/3	−	+ ↑	−	+ ↑
93727	51	M	3/3	−	+ ↓	−	−
94105	45	M	3/3	−	−	−	−
94364	47	F	3/3	−	+ ↓	−	−
94367	56	M	3/3	−	+ ↓	−	±
94417	60	M	3/3	−	+ ↓	−	−
94530	52	M	3/3	−	+ ↓	−	−
94684	67	M	3/3	−	+ ↓	−	−
94760	48	M	3/3	−	+ ↓	−	−
94939	44	M	3/3	−	−	−	± ↑
95094	52	F	3/3	−	−	−	−
95303	52	F	3/4	+ ↓	2+ ↑	2+ ↑	2+ ↑
95348	40	M	3/3	−	+ ↓	−	−
95475	42	F	3/3	−	−	−	−
95531	40	M	3/3	−	−	−	−
95639	41	M	3/4	−	−	−	−
95770	47	M	3/3	−	−	−	−

Table 1B. Western Blot Data on EIA-negative CA1 Specimens from Autopsy Cases at Tokyo Medical Examiner's Office

Case	Age	Gender	ApoE	Aβ40(BA27)		Aβ42(BC05)	
				4kd	8kd	4kd	8kd
92818	61	M	2/3	−	2+ ↑	−	+ ↑
93023	45	M	3/4	−	+ ↑	−	−
93026	57	F	3/3	−	2+ ↑	−	± ↑
93033	59	M	3/3	−	−	−	+ ↑
93137	59	M	3/3	−	−	−	−
93328	56	M	3/3	−	2+ ↑	−	+ ↑
93334	48	M	3/3	−	−	−	± ↑
93459	65	M	3/3	−	−	−	± ↑
93545	33	M	3/3	−	−	−	−
93570	52	M	3/3	−	−	−	−
93650	44	M	3/3	−	−	−	−
93727	51	M	3/3	−	−	−	−
94068	55	M	3/3	−	2+ ↑	−	± ↑
94105	45	M	3/3	−	−	−	−
94364	47	F	3/3	−	+ ↓	−	±
94367	56	M	3/3	−	+ ↑	−	2+ ↑
94417	60	M	3/3	−	−	−	−
94530	52	M	3/3	−	−	−	± 2.2
94651	69	M	3/3	−	−	−	± ↑
94684	67	M	3/3	−	+ ↑	−	± ↑
94760	48	M	3/3	−	+ ↓	−	−
94939	44	M	3/3	−	+ ↓	−	−
94977	66	M	3/3	−	−	−	−
95005	50	M	3/3	−	−	−	−
95094	52	F	3/3	−	−	−	± 1.8
95303	52	F	3/4	−	−	−	−
95348	40	M	3/3	−	−	−	−
95475	42	F	3/3	−	−	−	−
95531	40	M	3/3	−	−	−	−
95639	41	M	3/4	−	−	−	−
95770	47	M	3/3	−	−	−	−
95848	48	M	3/3	−	−	−	−

−, negative; ±, faint signal; +, definite signal; 2+, strong signal; ↓, below the quantitation range; ↑, beyond the linear range; *, pmol (Aβ monomer equivalent)/g wet weight.

Table 2. Western Blot Data on EIA-negative Prefrontal Cortex Specimens from Autopsy Cases at Gunma Cancer Center

Case	Age	Gender	Postmortem delay (h)	ApoE	Aβ40(BA27) 4kd	Aβ40(BA27) 8kd	Aβ42(BC05) 4kd	Aβ42(BC05) 8kd
2	60	F	2.8	3/3	±	−	−	+
3	56	M	0.8	3/3	−	−	−	+ 1.2*
5	66	M	2.0	3/3	−	−	−	+
6	71	M	0.8	3/4	−	± ↓	−	+ 3.3
10	70	M	2.0	3/3	±	−	−	+ 0.6
13	79	F	10.3	2/3	−	± ↓	−	+ 5.1
14	48	F	1.75	3/4	−	−	−	+ 1.8
20	44	M	7.7	3/4	−	−	−	−
21	70	M	1.5	3/3	−	−	± 4.0	+ 2.0
23	53	M	4.8	3/4	−	−	−	−
24	51	M	1.2	3/3	−	± ↓	−	−
25	69	F	10.0	3/3	−	−	−	+ ↓
26	55	M	2.0	3/3	−	−	−	−
27	65	M	7.5	3/4	−	−	−	−
28	68	M	2.0	3/3	−	± 3.5	−	+ 2.6
29	57	M	1.0	3/3	−	2+ ↑	−	+ ↓
31	75	F	2.0	2/3	−	± ↓	−	+ 2.3
32	49	M	1.5	3/3	−	± ↓	−	+ 1.3
36	73	M	8.0	3/3	−	−	−	+ 1.9
38	60	M	2.5	3/3	−	−	−	+ 1.3
39	40	F	8.4	3/3	−	−	−	+ 1.9
40	67	M	0.67	3/3	−	−	+	+ 2.0

−, negative; ±, faint signal; +, definite signal; 2+, strong signal; ↓, below the quantitation range; ↑, beyond the linear range; *, pmol/g wet weight.

Discussion

Although we were unable to microsequence the Aβ dimer for confirmation, it is most likely that this represents the same Aβ dimer that Masters and colleagues originally found in the purified amyloid core fractions[28] and Roher and colleagues later extensively characterized.[29] The latter group demonstrated that in contrast to dissociable synthetic Aβ, Aβ dimer does not assemble into amyloid fibril but generates granular particles. A further striking characteristic of the Aβ dimer is that in its presence, microglia kill neurons in culture.[29] Roher and colleagues also showed that such SDS-stable Aβ dimer is generated *in vitro* when synthetic Aβ1–40 or Aβ1–42 is incubated for a long time.[29,30] Presumably, the carboxyl third of Aβ is responsible for formation of SDS-stable dimer, because Aβ1–28 does not form SDS-stable dimer by prolonged incubation.[30] Thus formed, Aβ dimer is claimed to be stable in formic acid or guanidine thiocyanate.[29,30]

The unexpected finding that BAN50 or BNT77 cannot capture the dimer but 4G8 can raises the possibility that the 6~8-kd band represents the dimer of p3 (Aβ17–42). However, this is unlikely because the 6~8-kd band in a number of specimens examined was also labeled with BAN50 (the epitope is located in Aβ1–10; see Figure 1C). Apparently, this conflicts with its capturing characteristic in EIA. SDS-denatured Aβ dimers on the Western blot may not take the same conformation as those in diluted guanidine hydrochloride. Possibly, the latter solution leads SDS-stable Aβ dimers and dissociable Aβ to take more native conformations. Thus, the BAN50 immunoreactivity with the 6~8-kd band on the blot is presumably created by the use of SDS.

The carboxyl terminus of the SDS-stable Aβ dimer is not uniform; generally, a large proportion of the dimers reacts with BC05 but not with BA27, whereas a small proportion of the dimers is labeled with BA27 and not with BC05. Predominance of BC05 or BA27 immunoreactivity in each case appears to be unrelated to postmortem delay. When the dimer in a given specimen is reactive with both antibodies, usually a fast-migrating part of the band was BC05-reactive and a slow-migrating part was BA27-reactive (see Figures 1 and 2). Thus, apparently there are two forms of the SDS-stable Aβ dimer: species ending at Aβ40 or Aβ42 exist presumably as homodimer. At present we do not have a proper explanation for the presence of two Aβ dimers in EIA-negative brains. One possible explanation would be that the generated SDS-stable Aβ42 dimers are rapidly converted to Aβ40 dimers through cleavage with a specific carboxyl dipeptidase in

Table 3. Effects of Postmortem Delay on Aβ in PDAPP Mouse Brain

Postmortem delay (h)	Age (m)	EIA (pmol/g wet weight) Aβ40	Aβ42
0	9.7	↓	46
0	9.7	13	40
2	9.3	20	68
2	9.3	14	47
4	9.7	16	86
4	9.5	↓	100
6	9.7	16	110
6	9.5	↓	52
12	9.7	↓	55
12	9.7	↓	16
18	9.7	↓	99
18	9.5	16	17*

↓, <12 pmol/g wet weight. *This transgenic mouse developed the SDS-stable Aβ dimer 18 hours after death.

some brains, but very slowly in other brains. The activity of the carboxyl dipeptidase may be relatively high in the brain because a significant proportion of synthetic Aβ1–42 injected into rat brain is rapidly converted to Aβ40.[31] Related to this, one may point to the possibility that SDS-stable Aβ42 dimer is generated from a potential precursor, Aβ43 dimer, by the action of another carboxyl peptidase. However, we were unable to detect BC65 immunoreactivity on the blot (data not shown). We are still not certain about whether the formation of Aβ42 dimer is indeed age-dependent, whereas that of Aβ40 dimer is not. To clarify this point, a much larger number of cases must be carefully studied.

The presence of soluble SDS-stable dimer was previously reported in CSF,[32] and we have independently confirmed the presence of SDS-stable Aβ40 dimer in CSF by Western blotting in a substantial proportion of aged control subjects and AD patients (Shinkai Y, Morishima-Kawashima M, Arai H, Ihara Y, unpublished observations). This may suggest that only Aβ42 dimer, not Aβ40 dimer, is pathogenic. It was also reported that the soluble fraction of AD brain contains SDS-stable Aβ dimer and ApoE complexes.[33] On the other hand, some cultured cells (CHO cells) secrete SDS-stable Aβ dimer and trimer[34] and transfection of mutant presenilin 1 or 2 to the cells, compared with that of wild-type presenilins, enhances SDS-stable oligomerization of the secreted Aβ.[35,36] A remarkable characteristic of this *in vitro* phenomenon is the oligomerization of Aβ at nanomolar or subnanomolar concentrations of Aβ, very close to physiological concentrations of Aβ in the extracellular space.

Although we cannot exclude the possibility that SDS-stable Aβ dimer is produced within the cell and released, it is attractive to postulate that SDS-stable Aβ dimer is generated in the extracellular space of the brain from dissociable Aβ constitutively secreted from brain cells. It should be noted that Aβ40 (and presumably also Aβ42) exists as dimer under physiological conditions.[37,38] In this context, it is of particular interest that oligomerization of Aβ, including dimerization, is enhanced in conditioned culture media, and that the addition of Congo red blocks the oligomerization.[36] The conditioned media appear to contain a factor or factors that enhance oligomerization. Perhaps one of the factors is Aβ42 itself because the culture media of mutant APP or presenilin 1- or 2-transfected cells that enhance Aβ oligomerization are known to contain increased levels of Aβ42.[36]

Thus, it is possible that SDS-stable dimer and oligomer are generated from secreted dissociable Aβ in the extracellular space under influences of many factors. At the very initial stage of Aβ accumulation the concentrations of soluble SDS-stable Aβ dimer and oligomers may increase. This may be because of increased Aβ, particularly Aβ42, production and/or decreased Aβ degradation. As the clearance becomes more defective with age, soluble SDS-stable Aβ oligomers reach saturation level and is deposited, a step which makes clearance or elimination more difficult. An alternative but not mutually exclusive possibility is that SDS-stable Aβ dimer may have a stronger affinity to extracellular matrix in brain. Thus, the presence of SDS-stable Aβ dimer may reflect the unusually slow process in the Aβ accumulation in the human brain.

In the above context, it is particularly intriguing that a quite recent report describes the neurotoxicity of diffusible, nonfibrillar (SDS-stable) Aβ1–42 oligomers.[39] These oligomers are claimed to show potent neurotoxicity at nanomolar concentrations, through a particular (as yet unidentified) cell-surface receptor, and the activation of fyn, a protein tyrosine kinase of the src family.[39] Furthermore, it has been reported that incubation of rat hippocampal slices with these oligomers prevents long term potentiation before signs of neuronal degeneration appear.[39] Related to this, it is rather surprising to note that a substantial proportion of CA1 specimens similar to that of T4 specimens already contain SDS-stable Aβ dimers (Table 1A and B). This indicates that, although CA1 is the site least affected by the deposition of Aβ (SDS-dissociable Aβ),[7] SDS-stable Aβ dimers appear in CA1 as early as in T4, one of the most affected sites. This may further suggest that there are two stages for β-amyloid deposition in humans: initial deposition of SDS-stable Aβ dimers followed by SDS-dissociable Aβ. Presumably, subsequent deposition of SDS-dissociable Aβ is significantly delayed in CA1 for unknown reasons. If diffusible Aβ42 oligomers were indeed toxic, this might explain why a number of neurofibrillary tangles in CA1 are already present in the stage showing no accumulation of SDS-dissociable Aβ as judged by EIA (Funato H and Ihara Y, unpublished observations). Thus, the major issue is whether the very low levels of SDS-stable Aβ dimer in the transgenic mice is related to the absence of neuronal loss[6] that defines the degree of dementia in humans.[40] Taken together, the findings of the present study suggest the possibility that SDS-stable dimers play important roles in β-amyloidogenesis in aged human brain and the neurodegeneration of AD.

Note Added in Proof

The data on the specificity of BA27 have recently been published (Morishima-Kawashima M, Ihara Y, Biochemistry 1998, 37:15247–15253).

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References

1. Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberburg I, Little S, Masliah E, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B, Zhao J: Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature 1995, 373:523–527
2. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G: Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 1996, 274:99–102
3. Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold K-L, Mistl C,

Rothacher S, Ledermann B, Burki K, Frey P, Paganetti P, Waridel C, Calhoun ME, Jucker M, Probst A, Staufenbiel M, Sommer B: Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc Natl Acad Sci USA 1997, 94:13287–13292

4. Johnson-Wood K, Lee M, Motter R, Hu K, Gordon G, Barbour R, Khan K, Gordon M, Tan H, Games D, Lieberburg I, Schenk D, Seubert P, McConlogue L: Amyloid precursor protein processing and Aβ42 deposition in a transgenic mouse model of Alzheimer disease. Proc Natl Acad Sci USA 1997, 94:1550–1555
5. Masliah E, Sisk A, Mallory M, Mucke L, Schenk D, Games D: Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F β-amyloid precursor protein and Alzheimer's disease. J Neurosci 1996, 16:5795–5811
6. Irizarry MC, Soriano F, McNamara M, Page KJ, Schenk D, Games D, Hyman BT: Aβ deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. J Neurosci 1997, 17:7053–7059
7. Funato H, Yoshimura M, Kusui K, Tamaoka A, Ishikawa K, Ohkoshi N, Namekata K, Okeda R, Ihara Y: Quantitation of amyloid β-protein in the cortex during aging and in Alzheimer's disease. Am J Pathol 1998, 152:1633–1640
8. Jarrett JT, Berger EP, Lansbury PTJr: The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Biochemistry 1993, 32:4693–4697
9. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y: Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43). Neuron 1994, 13:45–53
10. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y: Amyloid β-protein (Aβ) deposition: Aβ42(43) precedes Aβ40 in Down's syndrome: Ann Neurol 1995, 37:294–299
11. Suzuki N, Cheung TT, Cai X-D, Odaka A, Otvos LJr, Eckman C, Golde TE, Younkin SG: An increased percentage of long amyloid β protein secreted by familial amyloid β protein precursor (βAPP717) mutants. Science 1994, 264:1336–1340
12. Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S: Increased amyloid-β 42(43) in brains of mice expressing mutant presenilin 1. Nature 1996, 383:710–713
13. Tomita T, Maruyama K, Saido TC, Kume H, Shinozaki K, Tokuhiro S, Capell A, Walter J, Grunberg J, Haass C, Iwatsubo T, Obata K: The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid β protein ending at the 42nd (or 43rd) residue. Proc Natl Acad Sci USA 1997, 94:2025–2030
14. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Pooraj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S: Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 1996, 2:864–870
15. Kosaka T, Imagawa M, Seki K, Arai H, Sasaki H, Tsuji S, Asami-Odaka A, Fukushima T, Imai K, Iwatsubo T: The β APP717 Alzheimer mutation increases the percentage of plasma amyloid-β protein ending at Aβ42(43). Neurology 1997, 48:741–745
16. Tokuda T, Fukushima T, Ikeda S, Sekijima Y, Shoji S, Yanagisawa N, Tamaoka A: Plasma levels of amyloid β proteins Aβ1–40 and Aβ1–42(43) are elevated in Down's syndrome. Ann Neurol 1997, 41:271–273
17. Tamaoka A, Odaka A, Ishibashi Y, Usami M, Sahara N, Suzuki N, Nukina N, Mizusawa H, Shoji S, Kanazawa I, Mori H: APP717 missense mutation affects the ratio of amyloid β protein species (Aβ 1–42/43, and Aβ 1–40) in familial Alzheimer's disease brain. J Biol Chem 1994, 269:32721–32724
18. Gearing M, Mori H, Mirra SS: Aβ-peptide length and apolipoprotein E genotype in Alzheimer's disease. Ann Neurol 1997, 39:395–399
19. Ishii K, Tamaoka A, Mizusawa H, Shoji S, Ohtake T, Fraser PE, Takahashi H, Tsuji S, Gearing M, Mizutani T, Yamada S, Kato M, St George-Hyslop PH, Mirra SS, Mori H: Aβ1–40 but not Aβ1–42 levels in cortex correlate with apolipoprotein E ϵ4 allele dosage in sporadic Alzheimer's disease. Brain Res 1997, 748:250–252
20. Nakabayashi J, Yoshimura M, Morishima-Kawashima M, Funato H, Miyakawa T, Yamazaki T, Ihara Y: Amyloid β-protein (Aβ) accumulation in the putamen, and mamillary body during aging, and in Alzheimer disease. J Neuropathol Exp Neurol 1998, 57:343–352
21. Asami-Odaka A, Ishibashi Y, Kikuchi T, Kitada C, Suzuki N: Long amyloid β-protein secreted from wild-type human neuroblastoma IMR-32 cells. Biochemistry 1995, 34:10272–10278
22. Shinkai Y, Yoshimura M, Morishima-Kawashima M, Ito Y, Shimada H, Yanagisawa K, Ihara Y: Amyloid β-protein deposition in the leptomeninges, and cerebral cortex. Ann Neurol 1997, 42:899–908
23. Ida N, Hartmann T, Pantel J, Schroeder J, Zerfass R, Foerstl H, Sandbrink R, Masters CL, Beyreuther K: Analysis of heterogenous βA4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive western blot assay. J Biol Chem 1996, 271: 22908–22914
24. Yanagisawa K, Odaka A, Suzuki N, Ihara Y: GM1 ganglioside-bound amyloid β-protein (Aβ): a possible form of preamyloid in Alzheimer's disease. Nat Med 1995, 1:1062–1066
25. Yamaguchi H, Sugihara S, Ogawa A, Saido TC, Ihara Y: Diffuse plaques associated with astroglial amyloid β protein, possibly showing disappearing stage of senile plaques. Acta Neuropathol (Berl) 1998, 95:217–222
26. Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990, 31:545–548
27. Mori H, Takio K, Ogawara M, Selkoe DJ: Mass spectrometry of purified amyloid β protein in Alzheimer's disease. J Biol Chem 1992, 267:17082–17086
28. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K: Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 1985, 82:4245–4249
29. Roher AE, Chaney MO, Kuo Y-M, Webster SD, Stine WB, Haverkamp LJ, Woods AS, Cotter RJ, Tuohy JM, Krafft GA, Bonnell BS, Emmerling MR: Morphology and toxicity of Aβ-(1–42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. J Biol Chem 1996, 271:20631–20635
30. Kuo Y-M, Webster S, Emmerling MR, Lima ND, Roher AE: Irreversible dimerization/tetramerization and post-translational modifications inhibit proteolytic degradation of Aβ peptides of Alzheimer's disease. Biochim Biophys Acta 1998, 1406:291–298
31. Shin R-W, Ogino K, Kondo A, Saido TC, Trojanowski JQ, Kitamoto T, Tateishi J: Amyloid β-protein (Aβ) 1–40 but not Aβ1–42 contributes to the experimental formation of Alzheimer disease amyloid fibrils in rat brain. J Neurosci 1997, 17:8187–8193
32. Vigo-Pelfrey C, Lee D, Keim P, Lieberburg I, Schenk DB: Characterization of β-amyloid peptide from human cerebrospinal fluid. J Neurochem 1993, 61:1965–1968
33. Permanne B, Perez C, Soto C, Frangione B, Wisniewski T: Detection of apolipoprotein E/dimeric soluble amyloid β complexes in Alzheimer's disease brain supernatants. Biochem Biophys Res Commun 1997, 240:715–720
34. Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB, Selkoe DJ: Aggregation of secreted amyloid β-protein into sodium dodecyl sulfate-stable oligomers in cell culture. J Biol Chem 1995, 270:9564–9570
35. Xia W, Zhang J, Kholodenko D, Citron M, Podlisny MB, Teplow DB, Haass C, Seubert P, Koo EH, Selkoe DJ: Enhanced production and oligomerization of the 42-residue amyloid β-protein by Chinese hamster ovary cells stably expressing mutant presenilins. J Biol Chem 1997, 272:7977–7982
36. Podlisny MB, Walsh DM, Amarante P, Ostaszewski BL, Stimson ER, Maggio JE, Treplow DB, Selkoe DJ: Oligomerization of endogenous and synthetic amyloid β-protein at nanomolar levels in cell culture and stabilization of monomer by Congo red. Biochemistry 1998, 37:3602–3611
37. Garzon-Rodriguez W, Sepulveda-Becerra M, Milton S, Glabe CG: Soluble amyloid Aβ-(1–40) exists as a stable dimer at low concentrations. J Biol Chem 1997, 272:21037–21044
38. Walsh DM, Lomakin A, Benedek GB, Condron MM, Teplow DB: Amyloid β-protein fibrillogenesis: Detection of a protofibrillar intermediate. J Biol Chem 1997, 272:22364–22372
39. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL: Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins: Proc Natl Acad Sci USA 1998, 95:6448–6453
40. Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Peterson RC, Parisi JE, Hyman BT: Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997, 41: 411–420