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Quantification of Modified Amyloid β Peptides in Alzheimer Disease and Down Syndrome Brains

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Abstract. To gain insights into the different forms of modified amyloid β peptides (Aβ) in the Alzheimer disease (AD) and Down syndrome (DS) brain, we used two-site ELISAs with antibodies specific for isomerized (i.e. Aβ with L-isoaspartate at positions 1 and 7) and pyroglutamate-modified (i.e. Aβ beginning with pyroglutamate at position 3) forms of Aβ to quantitate the levels of these different Aβ peptides in formic acid extracts of AD and DS frontal cortex. Despite variations in the proportions of distinct forms of Aβ in AD and DS frontal cortex, the major species of Aβ in these samples were AβN3(pyroGlu)-42 as well as Aβx-42 (where x is a residue at position 2 or less in Aβ), whereas isomerized Aβ was a minor species. Further, the levels of isomerized and pyroglutamate-modified forms of Aβ terminating at amino acid 42 were higher than those ending at amino acid 40. The abundance of the distinct forms of Aβ reported here in formic acid extracts of AD and DS frontal cortex suggests that these Aβ species could play important roles in the deposition of Aβ in AD and DS brains.

Key Words: Alzheimer disease; Amyloid β peptide; Down syndrome; Enzyme-linked immunosorbent assay; Modification; Senile plaque.

INTRODUCTION

Alzheimer disease (AD) is characterized pathologically by the massive deposition of amyloid β peptides (Aβ) as insoluble filaments that accumulate in vascular amyloid and in senile plaques (SP) of AD brains (1). Aβ is proteolytically cleaved from longer β-amyloid precursor proteins (βAPP), by as yet unidentified proteases termed the β- and γ-secretases (2). The deposition of Aβ is considered to be closely related to the pathogenesis of AD because (i) the accumulation of Aβ as diffuse plaques is the earliest pathological change in Down syndrome (DS) brains and (ii) missense mutations in the Aβ-flanking region of βAPP genes (3–5) as well as in presenilin genes (6–9) of some familial AD pedigrees cosegregate with disease and foster Aβ deposition by altering the processing of βAPP.

It has been shown that the forms of Aβ in amyloid deposits of the AD and DS brain are heterogenous at their carboxyl (C)- and amino (N)-termini (10–19). Aβ ending at residue 42 (Aβ42) aggregates much faster than Aβ ending at residue 40 (Aβ40) (20), and Aβ42 deposits initially and preferentially as amyloid (10–15). The N-terminus of Aβ also exhibits several different modifications including ragged N-termini (11, 15), pyroglutamation (16, 17), racemization and isomerization (10, 18, 19). For example, Aβ beginning with a cyclized glutamate (pyroglutamate) at position 3 (16–20) as well as Aβ with L-isoaspartate at positions 1 and 7 (10) have been demonstrated in SP amyloid by rigorous protein- and immunochemical studies. These modifications are of particular interest because they may contribute to the deposition of Aβ by hampering the proteolytic degradation of Aβ by aminopeptidases (16–19, 22) or by altering the conformation of Aβ and thereby foster deposition (22, 23). However, quantitative analysis of these modified Aβ species in amyloid deposits has not been performed yet. Moreover, the precise N- and C-terminal properties of the forms of Aβ that predominate in the amyloid deposits of the AD and DS brains remain to be elucidated. Here we report the establishment of two-site enzyme-linked immunosorbent assay (ELISA) systems that can selectively quantitate species of Aβ40 and Aβ 42 with isomerized or cyclized amino acids at their N-termini, respectively, and we found that AβN3(pyroGlu)-42 as well as Aβx-42 (where x is a residue at position 2 or less in Aβ) are the predominant species deposited as parenchymal amyloid.

MATERIALS AND METHODS

Peptides

Several synthetic peptides were used in this study, and [Cys[17]]-Aβ1-16 with L-isoaspartate at positions 1 and/or 7 (Aβ1-16[1,7diL-isoAsp], Aβ1-16[1L-isoAsp], Aβ1-16[7L-isoAsp]) as

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well as Aβ1-40[1,7diL-isoAsp] and Aβ1-42[1,7diL-isoAsp] were synthesized using an automated peptide synthesizer as previously described (17, 18, 23). Also, the peptides AβN3(pyroGlu)-40 and AβN3(pyroGlu)-42 with pyroglutamate at the N-terminus of Aβ3-40 and Aβ3-42, respectively, were similarly synthesized (17, 18, 23). Aβ1-40 and Aβ1-42 were obtained from Bachem Feinchemikalien AG (Bubendorf, Switzerland).

Antibodies

Five to 10 μg of Aβ1-16[1,7diL-isoAsp] conjugated with bovine thyroglobulin via Cys^{17} together with complete or incomplete Freund's adjuvant, were injected into the foot pads of BALB/c mice on day 1, 4 and 7. The popliteal lymphnodes were harvested on day 10 and fusion was performed as described (5). Hybridoma supernatants were loaded on microwell plates precoated with anti-mouse IgG/M antibodies together with HRP-conjugated Aβ1-16[1,7diL-isoAsp], and clones that showed strong peroxidase reaction were selected. Similarly, competitive ELISAs were performed in the presence of unbound Aβ1-16[1,7diL-isoAsp], Aβ1-16[1L-isoAsp], Aβ1-16[7L-isoAsp] or Aβ1-16 as competitors to examine the specificities of the monoclonal antibodies (mAbs).

BAN52 is a mouse mAb raised against Aβ1-16 that recognizes the N-terminus of full-length Aβ, especially those beginning at N1(L-Asp) (ref. 24 and see results). Anti-AβN3(pyroGlu) polyclonal antibody was raised against a synthetic peptide AβN3(pyroGlu)-7 and specifically recognizes the N-terminus of Aβ beginning at N3(pyroGlu) as described (17, 18). BA27 and BC05 are mouse mAbs that specifically react with the C-terminus of Aβ1-40 and Aβ1-42(43), respectively (5, 12–15).

ELISAs

The two-site ELISAs for Aβ were carried out as previously described (5, 12), with some modifications. Briefly, microwell plates were precoated with BA27 (30 μg/ml) or BC05 (15 μg/ml). Standard peptides or samples diluted with buffer EC[20mM phosphate, 400 mM NaCl, 2mM EDTA, 0.4% Block Ace (Snow Brand Milk Products, Sapporo, Japan), 0.2% bovine serum albumin, 0.05% CHAPS, 0.05% NaN_3, pH 7.0] were put in each well and reacted at 4°C overnight. After washing, loaded wells were reacted with HRP-labeled BAN52 or 1H10 (mAb specific to Aβ[1,7diL-isoAsp]; see Results) or with unbound anti-AβN3(pyroGlu) for 6h at 4°C. For anti-AβN3(pyroGlu), loaded wells were then reacted with HRP-labeled anti-rabbit immunoglobulin antibody for 6h at 4°C. Bound enzyme activity was measured by TMB microwell peroxidase system (Kirkegaard & Perry laboratories, Gaithersburg, Md.), and the levels of different Aβ species were normalized by the wet tissue weight of the starting materials.

Cases

Fourteen autopsied cases with sporadic AD (sAD) and 5 cases of DS with AD pathology were used in this study. Clinicopathological information on these cases is summarized in Table. A hemisphere from each brains obtained at autopsy was dissected into coronal slabs that were frozen and stored at −80°C until used in this study. AD was diagnosed using published clinical and pathological criteria (25, 26).

Extraction of Aβ from Brain Samples

Cortical tissues from middle frontal gyrus (Brodmann's area 8) were dissected and cortical gray matter and covering leptomeninges were carefully separated. Approximately 1 g of gray matter was minced with a scalpel blade and homogenized in 5 volumes of Tris-buffered Saline (TBS; 50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, protease inhibitors [0.1 mM diisopropylfluorophosphate, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml Nα-P-tosyl-L lysine chrolomethyl ketone, 1 μg/ml antipain, 0.1 μg/ml leupeptin]) by 10 strokes with a motor-driven Teflon homogenizer. The homogenates were spun at 500,000 × g for 20 min at 4°C. The pellets were resuspended in 5 volumes of TBS/protease inhibitors containing 1.0 mol/L sucrose and spun again at 500,000 × g for 20 min. The pellet was homogenized by 10 strokes with a Teflon homogenizer in 3 volumes of 1% TritonX-100/TBS/protease inhibitors, incubated for 15 min at 37°C, and spun at 500,000 × g for 20 min. The pellets were then homogenized in 3 volumes of 2% sodium dodesyl sulfate (SDS)/TBS/protease inhibitors, incubated for 15 min at 37°C, and spun at 500,000 × g at 25°C. The pellets were ultrasonicated in 1 ml of 70% formic acid and spun at 500,000 × g at 4°C. The supernatant was collected, desiccated in a Speed Vac, and resuspended in 100 μl of DMSO by a brief ultrasonication. Thus, formic acid-extracted Aβ prepared in this manner was preserved in DMSO at −80°C until used.

Immunocytochemistry and Morphometry

Small blocks of frontal cortex obtained from areas adjacent to those used for extraction were fixed in 10% neutral buffered formalin for 12 hrs at 4°C and 50 μm-thick sections were cut on a microslicer. Floating sections were pretreated with 90% formic acid for 5 min and immunostained with BAN52, 1H10, anti-AβN3(pyroGlu) by the standard avidin-biotin complex method using 3,3'-diaminobenzidine as chromogen as described (12, 13, 19). For the morphometric evaluation of the extent of the amyloid deposits, the percentage of a defined area covered by plaques positive for anti-AβN3(pyroGlu), which immunostained virtually all plaques of various types, was calculated in 3 unselected cortical areas (each of which was 1.2 mm^2) by computer-assisted morphometry as previously described (12, 13).

Immunoblot Analysis

Formic acid-extracted brain samples as well as synthetic peptides were dissolved in sample buffer containing 8 M Urea, heated for 3 min at 100°C, and separated by Tris/Tricine SDS-PAGE using a 10–16% gradient gel containing 4M Urea (17, 18). Chemiluminescence method was employed to visualize immunoreactive bands.

RESULTS

Characterization of the Aβ N-terminal Specific Antibodies and Establishment of Two-site ELISAs

We raised a mAb (1H10) that specifically reacts with the N-terminal portion of Aβ with L-isoaspartate at positions 1 and 7 (Aβ[1,7diL-isoAsp]). To examine the specificity of 1H10 against Aβ[1,7diL-isoAsp], 1H10 was coincubated with HRP-labeled Aβ1-16[1,7diL-

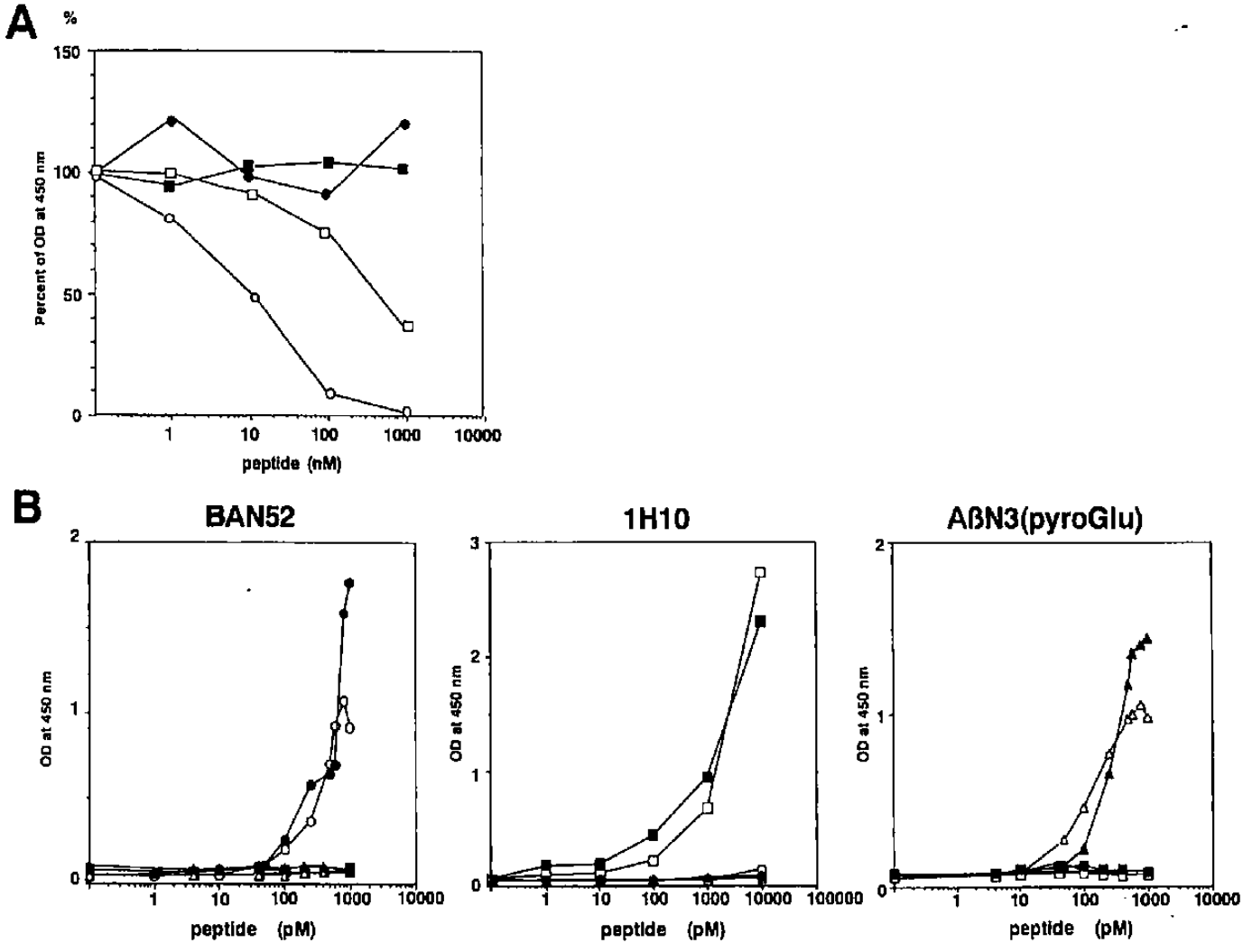

Fig. 1. Characterization of the Aβ N-terminal specific antibodies and ELISAs. A. Competitive ELISA analysis of mAb 1H10. 1H10 (~2 μg/ml) was coincubated with HRP-labeled Aβ1-16[1,7diL-isoAsp] on microplate wells precoated with anti-mouse IgG antibody, together with similar amounts of unbound Aβ1-16[1,7diL-isoAsp] (O), Aβ1-16[7L-isoAsp] (□), Aβ1-16[1L-isoAsp] (●) or Aβ1-16 (■). The percentage of optical densities against those obtained without unbound competitor peptides was plotted. B. Specificities and sensitivities of the two-site ELISAs. Each amount of Aβ1-40 (O), Aβ1-42 (●), Aβ1-40[1,7diL-isoAsp] (□), Aβ1-42[1,7diL-isoAsp] (■), AβN3(pyroGlu)-40 (△) and Aβ3pyroGlu-42 (▲) were placed on BA27- (for Aβ40) or BC05- (for Aβ42) coated plate. Bound antigen was detected by HRP-labeled BAN52 or 1H10, or by anti-AβN3(pyroGlu)/HRP-labeled anti-rabbit IgG.

isoAsp] on microplate wells precoated with anti-mouse IgG antibody, together with each of the unbound Aβ1-16 peptide with L-isoAsp or L-Asp at positions 1 and 7. Aβ1-16[1,7diL-isoAsp] most efficiently competed with the reaction of 1H10 with HRP-conjugated Aβ1-16[1,7diL-isoAsp],, and Aβ1-16[7L-isoAsp] competed very weakly (~100 fold less potent compared to Aβ1-16[1,7diL-isoAsp]), whereas Aβ1-16[1L-isoAsp] or Aβ1-16 did not compete (Fig. 1A). This suggested that 1H10 specifically recognizes the N-terminus of Aβ[1,7diL-isoAsp].

We then established 6 different ELISAs that distinguish Aβ40 and Aβ42 beginning at AβN1(L-Asp), Aβ[1,7diL-isoAsp] or AβN3(pyroGlu), respectively (Fig. 1B). ELISAs using 1H10 or anti-AβN3(pyroGlu) as detector antibodies were specific for Aβ[1,7diL-isoAsp] or AβN3(pyroGlu)Aβ, respectively. ELISAs using BA27 (or BC05) exclusively captured standard peptides ending at Aβ40 (or Aβ42), respectively, and never crossreacted with Aβ42 (or Aβ40) with the N-terminus of the same type (data not shown). When BAN52 was used as a detector antibody, Aβ1-40 (when BA27 was used as a capture antibody) or Aβ1-42 (when BC05 was used as a capture antibody) was selectively detected, whereas Aβ1-40 (or 1-42)[1,7diL-isoAsp] or AβN3(pyroGlu)-40 (or 42) were not detected (Fig. 1B). However, BA27/BAN52

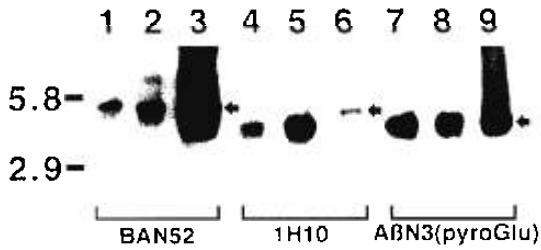

Fig. 2. Immunoblot analysis of Aβ with N-terminal specific antibodies. Twenty ng of synthetic Aβ1-40 (lane 1), Aβ1-42 (lane 2), Aβ1-40[1,7diL-isoAsp] (lane 4), Aβ1-42[1,7diL-isoAsp] (lane 5), AβN3(pyroGlu)-40 (lane 7) and Aβ3pyroGlu-42 (lane 8), as well as 1 μl (lanes 3 and 9) or 5 μl (lane 6) of Aβ extracted from frontal cortical parenchyma of an AD brain (case 12 in Table) and dissolved in DMSO (equivalent to ~80 and ~400 μg of Aβ peptides, respectively), were separated by SDS-PAGE and immunoblotted with BAN52 (lanes 1–3), 1H10 (lanes 4–6) and anti-AβN3(pyroGlu) (lanes 7–9). Arrows indicate the major 4 kDa (lanes 3, 6) or 3.7 kDa (lane 9) Aβ species derived from parenchymal amyloid. Molecular mass standards are shown in kilodaltons.

ELISA detected AβN(-3 or -6)-40 at similar specificities, and AβN1(D-Asp)-40 as well as AβN2(Ala)-40 at ~50% intensities compared to those for AβN1(L-Asp)-40 (data not shown); thus, BAN52 was used as an immunoprobe that collectively detects Aβ species beginning at residue x (where x is a residue at position 2 or less in Aβ).

Immunoblot and Immunocytochemical Detection of N-terminally Modified Aβ

We confirmed the presence of N-terminally modified Aβ in formic-acid extracts of AD and DS brains by immunoblot and immunocytochemical analyses. On immunoblots, BAN52, 1H10 and anti-AβN3(pyroGlu) specifically reacted with synthetic peptides of Aβ1-40 (or Aβ1-42), Aβ1-40 (or 42)[1,7diL-isoAsp], and AβN3(pyroGlu)-40 (or 42), respectively (Fig. 2), and never crossreacted with other sets of Aβ species with different N-termini. In the formic acid extracts of AD and DS brains, BAN52 reacted chiefly with a 4 kDa band (Fig. 2, lane 3, arrow) and anti-AβN3(pyroGlu) reacted with a slightly lower 3.7 kDa band (Fig. 2, lane 9, arrow), together with some high molecular weight smears. In contrast, 1H10 reacted with a 4 kDa band of relatively weaker intensity (Fig. 2, lane 6, arrow). By immunocytochemistry, BAN52 (Fig. 3A) and anti-AβN3(pyroGlu) (Fig. 3B) strongly immunolabeled virtually all plaques of various types, whereas 1H10 immunostained a small proportion of SP, especially the central cores of classical SPs (Fig. 3B, arrows). Peripheral portions of classical SPs (arrows) as well as a few primitive plaques (Fig. 3B, arrowhead) were weakly labeled by 1H10.

ELISA Quantification of Amino-terminally Modified Aβ in AD and DS Brains

We then quantitated the 6 different Aβ species extracted from AD and DS cortices using the two-site ELISAs

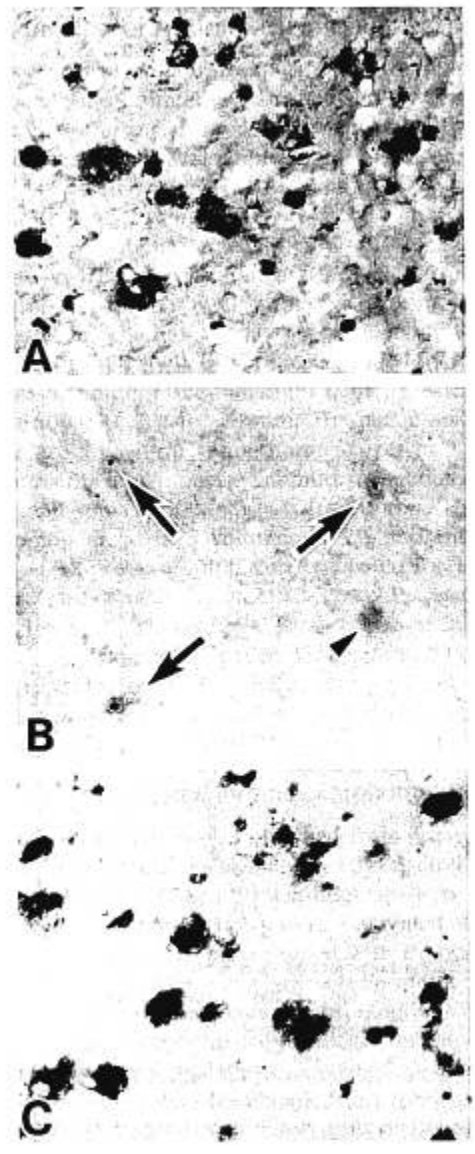

Fig. 3. Immunostaining patterns of senile plaques with Aβ N-terminal antibodies. BAN52 (A) and anti-AβN3(pyroGlu) (C) strongly immunolabeled all types of plaques, whereas 1H10 immunostained a small proportion of SP, especially the central cores of classical SPs (arrows, B). Peripheral portions of classical SPs (arrows) or occasionally, primitive plaques (arrowhead), were weakly labeled by 1H10. Frontal cortex of a 53-year-old DS patient (case 5 in Table). Magnification, ×83.

(Table). In the frontal cortex of AD brains, Aβ42 comprised the predominant Aβ C-terminal species, and the average level of AβN3(pyroGlu)-42 was the highest among these species, followed by that of Aβx-42 ($x < 3$) detected with BAN52. In contrast, the average level of Aβ1-42[1,7diL-isoAsp] was relatively lower, which amounted to ~20% of those of the latter 2 major species. Although the levels of different Aβ species showed marked variations between cases, the levels of all 3 Aβ42 species correlated positively with the percentage of a defined area stained by the anti-AβN3(pyroGlu) antibody

TABLE
Summary of Cases and Levels of Aβ in AD and DS Brains

Case no.	Age (year)	Sex	Duration (year)	PMI (hr)	apoE	SP %area	CAA	(μg/g Wet tissue weight) Aβx-40 (x < 3)	Aβ1-40 (1,7diL-isoAsp)	AβN3 (pyroGlu)−40	Aβx-42 (x < 3)	Aβ1-42 (1,7diL-isoAsp)	AβN3 (pyroGlu)-42
AD													
1	83	F	10	12	NE	9.6	−	4.21	0.00	0.47	0.60	0.02	2.06
2	83	F	3	4	NE	12.8	−	0.24	0.11	0.02	2.22	0.23	1.26
3	80	M	10	23	NE	12.5	−	0.23	0.02	0.01	2.62	0.23	1.40
4	86	F	7	5	3/3	13.5	−	0.35	0.001	0.11	1.94	0.55	5.02
5	68	M	5	10	4/4	15.6	−	4.11	0.01	0.35	3.77	0.37	7.29
6	80	M	8	36	3/4	26.1	−	2.86	0.11	0.48	7.64	1.79	8.12
7	84	F	6	12	3/3	8.6	−	0.03	0.01	0.00	1.60	0.001	0.85
8	82	M	7	11	3/4	19.5	+	2.26	0.03	0.23	3.43	0.71	2.98
9	66	F	2	18	3/4	10.5	−	0.01	0.00	0.01	1.20	0.30	0.75
10	76	M	6	24–48	3/4	12.8	−	3.65	0.11	0.03	2.10	0.30	2.66
11	68	F	4	18	2/4	20.7	+	4.58	0.05	0.21	7.24	1.65	6.61
12	69	F	7	26	3/3	6.9	−	0.62	0.02	0.02	3.55	0.81	2.94
13	75	F	12	48	3/3	12.8	−	0.04	0.001	0.01	2.64	0.93	1.63
14	82	M	8	48	3/4	18.4	−	0.044	0.00	0.04	1.81	0.11	2.99
Mean ± SE	76.4 ± 7.7							1.66 ± 0.50	0.03 ± 0.01	0.14 ± 0.05	3.14 ± 0.53	0.61 ± 0.14	3.18 ± 0.70
DS													
1	67	F	5	36	NE	15.1	−	5.71	0.16	0.94	1.84	0.49	24.00
2	61	F	5	5	NE	10.8	−	2.17	0.03	0.00	1.61	0.86	3.43
3	57	F	3	7.5	NE	9.0	+	3.45	0.84	2.50	7.91	0.54	4.48
4	54	M	3	14	NE	11.1	+	52.27	0.17	0.55	0.38	0.05	10.11
5	53	F	0	15	NE	9.9	−	0.50	0.00	0.00	6.28	1.01	7.00
Mean ± SE	59.0 ± 5.3							12.82 ± 9.90	0.24 ± 0.15	0.80 ± 0.46	3.60 ± 1.47	0.59 ± 0.17	9.80 ± 3.73

Levels of 6 different types of Aβ extracted from frontal cortices of individual cases, together with the average levels ± SE in AD and DS groups, are shown. Aβ levels are shown in μg/g wet tissue weight. AD: Alzheimer disease, DS: Down syndrome. Duration from clinical onset of dementia to death and postmortem intervals before autopsy (PMI) are shown in years and hours, respectively. ApoE genotypes verified by PCR amplification analysis of DNAs obtained from frozen brain tissues or paraffin sections are shown; NE: not examined. The percentages of the cortical areas covered by plaque amyloid (SP %area) were calculated as described in materials and methods, and cases which harbored more than 1 Aβ-positive parenchymal vascular amyloid deposits in an immunostained section (width: ~10 mm) were indicated as CAA (+).

(correlation efficiencies: r = 0.754 for Aβx-42 (x < 3), r = 0.674 for Aβ1-42[1,7diL-isoAsp] and r = 0.720 for AβN3(pyroGlu)-42). As regards Aβ40 in the brain parenchyma, Aβx-40 (x < 3) comprised the most predominant species, and the ratios of AβN3(pyroGlu)-40 or Aβ1-40[1,7diL-isoAsp] to Aβx-40 (x < 3) were lower (~8% and ~2%, respectively) compared to those in Aβ42. In the frontal cortex of DS brains, the predominance of AβN3(pyroGlu)-42 over Aβx-42 (x < 3) was more marked than in the AD samples. No correlation was found between the postmortem interval and the levels of different Aβ species (data not shown). Individual data on these samples are summarized in Table.

DISCUSSION

In this study, we raised antibodies that specifically recognize distinct modifications at the N-terminus of Aβ and established multiple types of two-site ELISAs that specifically quantitate Aβ40 or Aβ42 species beginning at N3(pyroGlu), 1,7diL-isoAsp at the N-terminus, respectively. We found that AβN3(pyroGlu)-42 as well as Aβx-42 (where x is a residue at position 2 or less in Aβ) were the predominant Aβ species in AD and DS frontal cortex. These peptides probably are derived chiefly from plaque amyloid because we carefully removed the leptomeninges and associated blood vessels from our samples, and parenchymal vascular amyloid deposits were observed in relatively small percentage of the cases studied here (Table). However, the possibility that some of these Aβ peptides are derived from microvascular deposits cannot be excluded. In addition, the levels of different Aβ species showed marked variations between cases. It has been shown that the levels of formic acid-extractable Aβ may exhibit profound variations (i.e. ~1,000 times) between cerebral cortices from AD cases harboring abundant plaques (15). The reasons for these marked individual variations are not clear at present, but it is plausible that the solubility of amyloid deposits varies due to different stages of plaque maturation, morphology or Aβ packing density in different individuals. Thus, several factors may influence the extent to which Aβ is extractable. However, we found that the levels of all 3 modified or unmodified Aβ42 species correlated positively with the amyloid burden (i.e. the percentage of the cortical area occupied by plaques), suggesting that the levels of extractable Aβ reflect the total of the amyloid deposits.

It has been repeatedly shown that Aβ42 is the predominant C-terminal species of plaque amyloid by immunocytochemistry (12–14, 19) and biochemistry (10, 11, 15), whereas the N-terminal properties of these Aβ42 species remained unknown. The predominance of AβN3(pyroGlu)-42 in parenchymal amyloid in AD and DS brains strongly suggests that the greater propensity of Aβ42 to aggregate and form amyloid is conferred by the longer C-terminus of Aβ42 (21), and that the truncated and cyclized N-teminus of Aβ may be less prone to attack by aminopeptidases (17, 18) thereby enhancing the aggregation (27) and deposition of this unique Aβ species. Taken together with the recent findings that AβN3(pyroGlu) is the predominant species in parenchymal amyloid of the brains of young DS patients containing exclusively Aβ42-positive diffuse plaques (18), and that a 3.7 kDa species of Aβ42 detected in the soluble fraction of DS brains prior to the deposition of insoluble Aβ (28) is positive for AβN3(pyroGlu) (29), it is conceivable that AβN3(pyroGlu)-42 as well as other Aβ42 species, could accumulate initially in the brain and trigger the formation of insoluble Aβ deposits.

The isomerized forms of Aβ (i.e. those with L-isoAsp residues at positions 1 and 7) were relatively minor species in parenchymal amyloid. This apparently does not agree with the earlier findings showing that the tryptic fragments of Aβ(6–16) derived from plaque amyloid contain a considerable amount of L-isoAsp at position 7 (10). This may be explained by the fact that N-terminally truncated Aβ, e.g. AβN3(pyroGlu), may harbor L-isoAsp at position 7, which fails to be detected by our antibody that reacts most strongly with Aβ[1,7diL-isoAsp]. Alternatively, L-isoaspartate O-methyltransferase, a repair enzyme of isomerized L-aspartyl residues, may minimize the amount of isomerized Aβ deposited as brain amyloid. Indeed, it has been shown that the lack of this enzyme causes serious brain damage leading to fatal seizures (30, 31). Further immuno- and protein chemical characterization will be needed to correctly evaluate the extent of isomerization of the Asp residues in Aβ deposited in brain amyloid. The selective localization of Aβ[1,7diL-isoAsp] in the cores of mature plaques may indicate that the conformational changes of Aβ due to the alterations in peptide backbone caused by isomerization (10, 22, 23) could contribute to the susceptibility of Aβ to form stable amyloid cores.

The extent of N-terminal modification due to pyroglutamation and isomerization was larger in Aβ42 compared to Aβ40. Since the deposition of Aβ42 precedes that of Aβ40 by more than 10 years in DS brains (13), these modifications might have occurred in Aβ42 that was deposited for a longer period of time compared to Aβ40, i.e. due to "aging" of these peptides. Alternatively, it is possible that these modifications are catalyzed enzymatically, and if so, these enzymes could be potential targets of therapeutic agents designed to reduce amyloid deposition. It should also be determined if these modifications occur before or after the deposition of Aβ. Further investigations into the posttranslational modifications of Aβ in brain amyloid will facilitate the understanding of Aβ-amyloidogenesis and the development of therapeutic strategies to decrease Aβ deposition in AD and DS.

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