E-a) Ch-, GM1-free at pH 7.4
i)   ii)   iii)

E-b) Ch-, GM1-free at pH 5.5
i)   ii)   iii)

FIG. 4—*continued*

F) CCM at pH 7.4
i)   ii)   iii)

G) Ch-, GM1-free at pH 7.4
i)   ii)   iii)

free membranes, both fibrils and spherical liposomes were present after 1 day, although with time the number of fibrils increased and the spherical liposomes disappeared (Fig 4G). Similar images (data not shown) were observed for the Ch-free membranes.

DISCUSSION

Because Aβ peptides are generated by the partial processing of the transmembrane α-helix of APP anchored in the brain membrane, their release from the membrane must play an important role in their subsequent aggregation and precipitation. Thus, to investigate how membrane lipids participate in the formation of fibril structure, we monitored the release of Aβ peptide from Aβ peptide-model membranes. Liposomes were prepared from Aβ and a lipid-mixture similar in composition to that of cerebral cortex membranes with and without ganglioside and/or cholesterol.

Our CD studies have shown that when a 50 μM solution of Aβ-(1–40) in HFIP solution is hydrated in buffer solution, it undergoes a transition from random coil to β-structure over a period of 3 days (Fig. 1A). This is consistent with the turbidity measurements. Over 24 h, the turbidity did not change, but it rapidly increased from 1 to 3 days, and only moderately after 3 days (Fig. 3A). From these results we propose that hydration of monomeric Aβ-(1–40) in organic solvent caused a slow change in conformation from random coil to β-structure. This β-struc-

ture then serves as a seed in the rapid formation of an extensive β-sheet structure (28). TEM measurements supported the presence of an extended fibril formation just 3 days after peptide hydration.

In CCM membranes at physiological pH (7.4), Aβ-(1–40) was mainly random coil after preparation, although some α-helical and β-sheet content was present (Fig. 1B). After 1 day, mainly β-structure had formed which persisted for 10 days. The turbidity increased moderately only for high concentrations of Aβ-(1–40) (Fig. 3B-a). TEM images of fibrals seen after 1 day (Fig. 4B-a, i) were clearer than those observed for the buffer solution. At pH 5.5 (Fig. 4B-b, i) the presence of fibrils after 1 day is uncertain, although after 3 days an extensive fibril formation is apparent (Fig. 4B-b, ii). For the CCM liposomes at endosomal pH the rate of formation of β-structure and fibrils is definitely slower than at physiological pH; however, the final resulting fibril structures appear the same. That Aβ fibril formation is pH-dependent has been reported in the literature (43).

From the CCM liposomes Aβ-(1–40) was released rapidly, and resulted in the formation of a fibril structure. In contrast, in GM1-free liposomes, Aβ-(1–40) consisted of a mixture of α-helix and β-structure and with time the proportion of β-structure increased. However, a slight increase in turbidity was observed for 40 μM Aβ. Its TEM image showed at first an incomplete short fibril structure around the lipid surface, and in time, small vesicles began to emerge, until finally only aggregates of small vesicles were visible (Fig. 4C). Apparently, Aβ was able to solubilize the ganglioside-deficient membrane into small vesicles.

In Ch-free Lipid liposomes, a similar change from α-helix to β-structure was observed. The turbidity decreased drastically for 20 μM Aβ, but not so for 40 μM. However, there were few signs of fibril structure after 1 day; instead spherical liposomes were visible (Fig. 4D, i). From 3 to 14 days, a gradual thickening and elongation of the thin, short fibrils around the spherical vesicles took place (Fig. 4, D, ii and iii). These fibrils were different in appearance to those observed in the buffer and CCM membranes. We propose these were peptide-phospholipid membrane complexes, because we have shown previously that highly hydrophobic peptides can form nanotubular fiber structures (29–31). Ch may assist in fibril formation, by promoting the release of Aβ-(1–40) from natural membranes. Interestingly, in both GM1- and Ch-free liposomes, the CD spectra show a shallow minimum around 223 nm (Fig. 1D), which was not seen for the CCM and GM1-free liposomes. Moreover, the TEM showed liposomes of various shapes and sizes, but no fibril structure was seen. These phenomena were the same at neutral and acidic pH membrane solutions. This suggests that the coexistence of Ch and ganglioside in the CCM membrane has a crucial role in the release of Aβ-(1–40) from the membrane and the subsequent formation of fibril structures.

A recent study has shown that in different lipid membranes Aβ can follow two pathways of assembly: pathway 1) the formation of fibril structure in the presence of acidic lipids; and pathway 2) the formation of small aggregates (but no fibril structures) in the presence of neutral lipids (32).

This study shows GM1-free membranes promote the formation of β-structure and small peptide-lipid vesicles, several ten-fold Angstrom in diameter. This process may take pathway 2; the absence of GM1 leads to the decrease in the acidity of the membranes, resulting in the elimination of Aβ-fibril formation. However, membrane disruption still occurs via the expansion of aggregated Aβ through the bilayers, resulting in the solubilization of membranes to form small peptide-lipid vesicles. Matsuzaki and Horikiri (33) reported that Aβ has a high affin-

ity for GM1 ganglioside in the bilayer and is able to form a β-sheet structure. It has been reported that the tight binding of Aβ is to the sialic acid group of the GM1 (34). In the presence of GM1, Aβ probably follows pathway 1, a conformational transition from α-helix to a β-structure leading to fibril formation. However, tight binding of the peptide to GM1 may prevent its release from the membrane, resulting in accumulation of peptide and formation of a β-sheet scaffold structure. This may be the critical nucleus for fibril formation. After nucleation, fibril growth through the lipid bilayer results in destabilization of the membrane, leading to amyloid deposition or the formation of lipid particles (to be described below).

Ch promotes fibril structure formation as we observed by TEM; in the absence of Ch, a well defined fibril structure was not visible even after a few days. Interestingly, in the absence of Ch or GM1 Aβ does form a β-structure, so the formation of a β-structure does not necessarily lead to fibril formation. An increase in Ch in the membrane results in increased membrane stiffness and a decrease in membrane fluidity. Increased Ch content inhibits the insertion of Aβ into the membrane, resulting in an increase in Aβ concentration at the membrane surface and concomitant enhancement in the rate of Aβ fibrillogenesis (5). Therefore, the absence of Ch in the membrane will increase its fluidity, and so facilitate the insertion of the hydrophobic part of Aβ into the membrane (25). In Ch-free liposomes, the accumulation of Aβ into the membrane leads to its solubilization, resulting in the slow formation of thick fibril structures.

In the absence of both Ch and GM1, the characteristic CD pattern of β-structure with a negative at around 116 nm was not seen, and no fibril structure was observed by TEM. This suggests the coexistence of Ch and GM1 in the CCM membrane promote fibril formation. A strong decrease in turbidity at 40 μM Aβ was also observed. All of these results suggest that the Ch-GM1-free membrane was solubilized by Aβ (1–40). Yanagisawa et al. (6) have shown that GM1 ganglioside-bound amyloid β-proteins are a possible form of preamyloid in AD. They also reported that oligomeric Aβ can promote the release of lipid from neurons to form Aβ-lipid particles consisting of Ch, GM1, phospholipid, and Aβ. Recent model membrane studies using liposomes consisting of GM1, Ch, and sphingomyelin showed that an increase in GM1 as well as Ch changes the binding capacity of Aβ (7, 35). Our data indicate that GM1 and Ch strongly participate in the release of Aβ from the membrane and therefore are instrumental in amyloid precipitation.

It has been suggested that sphingolipids and cholesterol may exist as phase-separated "rafts" in sphingolipid and cholesterol-rich membranes such as the plasma membrane (36). The partial liquid-ordered rafts can be visualized as floating within the predominantly liquid crystalline "sea" of the lipid bilayer. Interestingly, it was proposed that the raft could be the site for the proteolytic processing of Alzheimer's amyloid precursor protein (APP) (37). Recently, we showed that elevating levels of sphingolipid and Ch cause decreased membrane fluidity and resulted in lipid-protein separations into liposomes containing α-helical transmembrane peptides (38). The proteolytic cleavage of APP to yield Aβ performed by both β-secretase and γ-secretase present in the raft may in fact involve the release of Aβ-lipid particles consisting of Ch, GM1, and phospholipid (8). However, in Ch- and GM1-free membranes, which are more fluid, Aβ is able to stay in the membrane, probably by insertion of its hydrophobic part into the lipid bilayer. The accumulation of Aβ in the membrane may result in its solubilization and eventual disruption into small vesicles.

The CCM consists of about 10% (w/w) plasmalogen (24), but the instability of this component under acidic conditions prevented its use in this study. We note, however, that it has been

reported in the literature that levels of plasmalogen in AD CCM are reduced (39, 40).

Aβ-(1–42) has been recognized to be the more amyloidogenic component in plaques, since it has a greater propensity to form β-structure than Aβ-(1–40), a requirement for amyloid fibral formation. Consequently, aggregates of Aβ-(1–42) may act as an initiation factor for early plaque formation (10). In the present study, Aβ-(1–40)/-(1–42) (10:1, molar ratio) formed β-structure more readily than Aβ-(1–40) in all classes of liposome. Especially, in Ch-GM1-free membranes, where Aβ-(1–40) forms no β-structure, the mixture of Aβ-(1–40)/-(1–42) caused the gradual conversion of a predominantly α-helical structure into a mainly β-structure. Therefore, the presence of a small amount of Aβ-(1–42) can induce the formation of β-structure in Aβ-(1–40) and confirms the "seeding" hypothesis. Aβ-(1–42) is able to promote the formation of β-structure that accompanies the formation of fibrils.

Recent studies have demonstrated that amyloid plaque formation may be initiated in the plasma membrane and that deposits were associated with the extracellular leaflet of the plasma membrane (41, 42). Moreover, Aβ-amyloid peptides are generated from various intracellular compartments, including the endoplasmic reticulum, the Golgi apparatus, lysosomes, and endosomes. The present model studies are carried out at the extracellular and lysosomal/endosomal pH values of 7.4 and 5.5. The results for the CCM liposomes indicate a kinetic pH-dependence of fibril formation, but it is not clear whether the release of Aβ-(1–40) is also pH-dependent. The results seen for the Ch-GM1-free liposomes are the same in both pH environments; fibril formation does not occur. This definitely indicates that lipid bilayer composition plays an important role in the release of Aβ and might suggest that this release is much less dependent on the pH of the surrounding cytosol.

It has been shown that ganglioside and Ch participate in the mechanism of amyloid deposition in the presence of total brain lipid extract (5, 11, 34). However, until now there has been no report in the literature on the behavior of Aβ in membranes free of both Ch and GM1. We have shown that Ch and GM1 play an important role in the release of Aβ from liposome membranes designed to model cerebral cortex membranes, where fibril structure formation is known to be at its highest. In natural brain membranes, the Aβ generated from the processing of APP may be easily released from the membrane to play its correct biological role. However, the change of lipid composition in membranes by aging or other biological processes induces Aβ accumulation in membranes, this leads to formation of amyloid fibers or lipid-peptide particles, which are released into the cytosol, resulting in amyloid precipitation or cytotoxicity.

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Eight-residue Aβ peptides inhibit the aggregation and enzymatic activity of Aβ42

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Abstract

Insoluble Aβ1–42 is the main component of the amyloid plaque. We have previously demonstrated that exposure to low pH can confer the molten globule state on soluble Aβ1–42 in vitro [Biochem. J. 361 (2000) 547] and unfolding experiments with guadinine hydrochloride (GdnHCl) have now confirmed this observation. The molten globule state of the protein has many biological properties and understanding the mechanisms of its formation is an important step in devising a therapeutic strategy for Alzheimer's disease (AD). We therefore investigated the ability of a series of synthetic eight-residue peptides derived from Aβ1–42 to inhibit the acid-induced aggregation of Aβ1–42 and identified the potent peptides to be Aβ15–22, Aβ16–23 and Aβ17–24. A1-antichymotrypsin, a member of the serine proteinase inhibitor (serpin) family is another major component of the amyloid plaque. In the present study, we investigated the proteolytic activity of Aβ1–42 against casein at different pHs. Chemical modification of amino acid residues in Aβ1–42 indicated that serine and histidine residues, but not aspartic acid, are necessary for enzymatic activity, suggesting that it is a serine proteinase. Amino acid substitution studies indicate that glutamic acids at positions 11 and 22 participate indirectly in proteolysis and we surmise that amino acid residues 29–42 are required to stabilize the conformer. A study of metal ions suggested that Cu^{2+} affected the enzymatic activity, but Zn^{2+} and Fe^{2+} did not. Interestingly, Aβ14–21 and Aβ15–22 were the only peptides that inhibited the proteolytic activity of Aβ42. Therefore, Aβ15–22 may control both aggregation of Aβ1–42 at acidic pH and its proteolytic activity at neutral pH. Consequently, we suggest that it may be of use in the therapy of Alzheimer's disease.
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Keywords: Alzheimer's disease; Serine-like proteinase activity; Proteinase inhibitor; Guadinine hydrochloride; Metal ions

1. Introduction

The deposition of amyloid plaque in the extracellular space, and neurofibrillary tangles in the neurons, are specific pathological features of Alzheimer's disease (AD) [1]. The main component of the amyloid plaque is insoluble Aβ1–42 (Aβ42), which adopts a structure rich in antiparallel β-pleated sheets. There have been many studies of the biological properties of Aβ42 such as self-aggregation [2], binding to other proteins such as apolipoprotein E [3], cytotoxicity for neuronal cells [4,5], vasoconstriction [6] and proteolytic activity against casein [7]. Recently, increasing awareness of amyloid beta protein (Aβ) intermediates as molten globule states has paralleled insight into the biological activities of the Aβ conformer [8,9]. The molten globule state of Aβ42 displays a less ordered, metastable conformation that is stabilized by the formation of fibrils [10,11]. Elucidation of the initial events causing aggregation and conversion of the soluble Aβ peptide into insoluble conformers, and other factors influencing the molten globule states of the Aβ conformer, are important aspects of a therapeutic strategy for AD.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; AD, Alzheimer's disease; Aβ, amyloid beta protein; GdnHCl, guadinine hydrochloride; EDTA, ethylene diamine tetraacetate; DEPC, diethyl pyrocarbonate; PK, proteinase K; PMSF, phenylmethylsulfonyl fluoride; DFP, di-isopropylfluorophosphate; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy) propane.

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We have already demonstrated that the conformation of Aβ42 varies at different pHs, that the critical pH for the change is 4.6, and that the conformer has a different susceptibility to proteinase K within the environment of a glial cell [12]. We therefore suggested that lowering the pH might generate the molten globule state of soluble Aβ42 in vitro. In the present study, we have performed unfolding experiments with acid-treated Aβ42 intermediates, using guadinine hydrochloride, and have confirmed our previous results. It has been reported that the Aβ peptide exists either mainly as a random coil/α-helical structure or a β-sheet structure depending on pH [2], and that the kinetics of aggregation depend on which of these structures is involved, as well as on the length of the peptide examined [13,14]. The aggregation process is known to be driven by hydrophobic interactions [15]. Hence, the adoption of a structure rich in β-sheet at acidic pH accelerates the aggregation of Aβ, and the abnormal Aβ is stabilized by intermolecular interactions with other Aβ monomers that have the ability to form a β-sheet conformation [16].

We have synthesized a series of partial eight-residue peptides derived from Aβ42 to study their ability to inhibit acid-induced aggregation of Aβ42. The common feature of amyloidosis-related disorders is the abnormal folding of a natural protein into a pathologic conformer that is proteinase resistant [17]. We therefore also investigated the ability of the short peptides to reverse the proteinase K susceptibility of Aβ aggregates in glial cells. Our previous data indicated that epitopes around residues 9–14 and 17–21 in Aβ42 were dramatically affected by acidic pH [12] and our present results indicate that peptides in the vicinity of Aβ15–22, which contain the central hydrophobic region of Aβ42, are able to interfere with pH-induced Aβ42 aggregation. Small peptides of this kind may be useful for preventing the conformational changes leading to formation of Aβ intermediates in the early stages of Aβ aggregation.

Aβ has been shown recently to possess proteolytic activity against casein [7] and this novel activity may be implicated in the mechanisms of AD. It has also been reported that an A1-antichymotrypsin that belongs to the serine proteinase family of inhibitors (serpins) is a major additional component of the amyloid plaque [18,19]. The serum level of this inhibitor increases in response to the acute phase reaction of the host defence system [20,21]. It is mainly synthesized in the liver, although also produced by astrocytes [22], and may reduce the toxicity of the amyloid peptides in clonal cell lines as well as in cultures of primary cortical nerve cells by inhibiting their proteinase activity [23]. Thus, inhibition of the proteolytic activity of Aβ may be a useful approach for preventing cellular toxicity. In the present study, we investigated the proteolytic activity of Aβ42 and identified essential amino acid residues and further investigated effects of bioessential metal ions for the activity. We also investigated the ability of eight-residue peptides to inhibit proteolysis at neutral pH and found that short peptides around Aβ15–22 are effective.

2. Materials

2.1. Aβ peptides

Fig. 1 shows the synthetic partial-length Aβ peptides used in the present study: Aβ1–16, Aβ1–28, Aβ12–28, Aβ17–42, Aβ1–40 (Aβ40), Aβ1–42 (Aβ42) and two Aβ1–40 derivatives, namely Aβ40 (E11Q) and Aβ40 (E22Q) in which glutamic acid at either position 11 or 22 is replaced by glutamine. These were purchased from Anaspec (San Jose, CA). All Aβ peptides except for Aβ17–42 were dissolved in water at 1mg/ml. Aβ17–42 was dissolved in dimethyl sulfoxide (DMSO). Peptides were used at the indicated concentrations.

2.2. Chemicals

Rink Amide MBHA resin and all the protected amino acids were purchased from NovaBiochem (Switzerland). Bovine serum albumin (BSA), proteinase K (PK) and phenylmethylsulfonyl fluoride (PMSF) were from Boehringer Mannheim (Germany). Guadinine hydrochloride (GdnHCl), disodium hydrogen phosphate, citric acid, Tween-20, Tris[hydroxymethyl]-aminomethane and di-iso-

Fig. 1. Synthetic partial length Aβ peptides.

propylfluorophosphate (DFP), ethylene diamine tetraacetate (EDTA), diethyl pyrocarbonate (DEPC) and 1,2-epoxy-3-(p-nitrophenoxy) propane (EPNP) were from Sigma-Aldrich (Tokyo, Japan). EnzCheck was purchased from Molecular Probes (Eugene, USA).

2.3. Antibodies

Monoclonal 4G8 antibody was from Signet Pathology Systems (Dedham, MA). 6F/3D (monoclonal) was from DAKO (Glostrup, Denmark) and anti 5–10 (monoclonal) was from QCB (Camarillo, CA). The epitopes recognised by the monoclonal antibodies were previously identified [12]: amino acid residues 17–21 (4G8), amino acid residues 9–14 (6F/3D) and amino acid residues 5–10 (anti 5–10 antibody). Alkaline phosphatase-conjugated, goat anti-mouse IgG from Promega (Madison, WI) was used as secondary antibody.

3. Methods

3.1. Denaturation of pH-modified Aβ42 with GdnHCl

Aβ42 (4 µg/ml) was incubated in either pH 4.6 or 7.4 buffer in Eppendorf tubes at 4 °C for 24 h and then plates were coated with 50 µl/well of solution for a further 24 h at 4 °C. After removing excess sample, 50 µl of GdnHCl was added to each well at the indicated concentrations and incubated for 30 min at 37 °C. After discarding the GdnHCl, the wells were washed three times with pH 7.4 buffer and the samples tested for reactivity with 6F/3D (1 µg/ml) using a standard enzyme-linked immunosorbent assay (ELISA) as described below.

3.2. Synthesis of the eight-residue peptides derived from Aβ42

A series of 8-mer peptides representing residues 1–32 of Aβ42 (overlapping by seven residues at the N terminus) were synthesized manually using solid phase peptide chemistry with Fmoc-protected amino acids on Rink Amide MBHA resin as previously described [12]. They were dissolved in water as indicated and used to inhibit Aβ42 aggregation and the proteolytic activity of Aβ42 for casein. Some of the peptides, Aβ16–23, Aβ17–24, Aβ18–25 and Aβ25–32, were dissolved in a minimal amount of DMSO before dilution with water.

3.3. Incubation of Aβ42 with the eight-residue peptides

Samples of Aβ42 (4 µg/ml) were mixed with each of the peptides (4 µg/ml) and incubated for 24 h at 4 °C in Eppendorf tubes in pH 4.6 or 7.4 buffer. The buffer used was 20 mM citric acid adjusted with disodium hydrogen phosphate.

3.4. Digestion of immobilized protein by proteinase K

Microtiter plate wells were coated with 50 µl of the mixtures described above for 24 h at 4 °C, and after removal of excess sample, the sample in each well was incubated with 50 µl of PK at 10 µg/ml in TBS, pH7.4 for 90 min at 37 °C. After removal of the proteinase K solution, the reaction was stopped by washing the well with TBST (TBS with 0.1% Tween-20, pH 7.4) followed by incubation with 3 mM PMSF at room temperature for 30 min, and rinsing twice with TBST. The samples were subsequently processed by standard ELISA assay as described below, to determine the amount of protein remaining.

3.5. ELISA assay

Samples after PK digestion were first incubated with TBS containing 3% BSA (pH 7.4) for 2 h at 37 °C, washed with TBST buffer and incubated for a further 2 h at 37 °C with 50 µl of anti 5–10 antibody (1 µg/ml) in TBS containing 1% BSA, pH 7.4. The wells were then washed with TBST, and incubated for an hour at 37 °C with 50 µl of a 1:5000 dilution of alkaline phosphatase-conjugated secondary antibody. After washing with TBST, bound antibody was detected by addition of p-nitrophenyl phosphate, and measured at 405 nm using a spectrophotometric plate reader (Molecular Devices) after 30 min. All washing steps were performed six times using a microplate autowasher, Model EL404 (BIO-TEK Instruments).

3.6. Cell line

The human glial cell line, KG-1-C, was obtained from RIKEN cell bank (Tsukuba, Japan) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and antibiotics at 37 °C in a humidified atmosphere containing 5% CO_2.

3.7. Cell culture with Aβ42 and peptides

Twenty-four-well flat-bottomed multiplates (Sumitomo Bakelite, Tokyo, Japan) were pre-coated with 100 µl per well of a mixture containing Aβ42 (4 µg/ml) and a short peptide (4 µg/ml), in either pH 4.6 or 7.4 buffer for 24 h at 4 °C. After removal of excess sample, wells were washed twice with phosphate-buffered saline (PBS), and 1.5 ml cells (2×10^4 cells/ml) was added per well, and incubated for 7 days. The cells were collected by scraper in 1 ml of PBS, adjusted to a density of 2×10^4 cells/ml in PBS and sampled for cell western dot blots.

3.8. Cell dot blots

One hundred microliters of each cell suspension was applied to a Dot Plate, DP-48 (Advantec, Tokyo, Japan) and blotted onto a methanol-immersed polyvinylidene difluoride

(PVDF) membrane (0.45 μm; Millipore, Bedford, MA) with absorption by vacuum pump.

The PVDF membrane was then removed, thoroughly air dried, and rinsed in TBS.

3.9. Digestion of samples with proteinase K

For digestion of the cells with proteinase K, the PVDF membrane were incubated with 5 ml of proteinase K at 10 μg/ml in TBS, pH 7.4 for 90 min at 37 °C with constant shaking. After removal of the proteinase K solution, the reaction was stopped by washing the membrane with TBST followed by incubation with 3 mM PMSF at room temperature for 30 min and rinsing twice with TBST. The membranes were subsequently processed for standard western blotting using 4G8 antibody to detect the protein remaining in the cell after PK digestion.

3.10. Cell western blot

PVDF membranes were blocked with 3% nonfat dry milk in TBST for 4 h, washed three times with TBST, and incubated with primary antibody 4G8 (1:10,000 dilution in TBS) for 2 h at 37 °C. This was followed by reaction with the secondary antibody, peroxidase linked anti-mouse IgG (1:5000 dilution in TBS) for 1 h at 37 °C. After three washes with TBST, the spots were detected by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech., Uppsala, Sweden) according to the manufacturer's instruction.

3.11. Assay of hydrolytic activity of Aβ peptides

The hydrolytic activity of Aβ peptides for casein was assayed using EnzCheck. Enhancement of fluorescence was observed as a result of casein cleavage products. Activity was detected with a pH-insensitive green fluorescent BODIOY FL casein substrate according to the manufacturer's instructions with excitation/emission maxima of 485/538 nm (Perkin-Elmer LS-5B spectrofluorometer). The fluorescence intensity of samples after incubations was corrected with solvent and expressed as relative fluorescence intensity. The dose-dependence of the enzymatic activity of Aβ42 at pH 7.4 was examined after 60-min incubation with the indicated concentrations of Aβ42. We also examined the activity of Aβ42 (5 μM) and Aβ17–42 (5 μM) as a function of incubation time at pH 7.4 and 37 °C. In another experiment, we compared the activity of Aβ1–16, Aβ1–28, Aβ12–28, Aβ17–42, Aβ40 and Aβ42 each at 5 μM and pH 7.4. The effect of pH 7.4 versus pH 4.6 was also tested for Aβ42, Aβ17–42 and Aβ1–16.

3.12. Incubation of Aβ42 with metals

The Aβ42 (2.5 μM) was mixed with $CuCl_2$, $ZnCl_2$ and $FeCl_2$ at 25 μM, respectively and incubated at 37 °C for 1 h,

and then tested for enzymatic activity using EnzCheck to study the effects of metal ions on the proteolytic activity of Aβ42 for casein. EDTA (50 μM) was also tested to reverse the effect of Cu^{2+} on the enzymatic activity.

3.13. Chemical modification of amino acid residues

Some of the amino acid residues of Aβ40 were modified by exposure to chemical compounds; di-isopropylfluorophosphate (DFP) for serine residues, diethyl pyrocarbonate (DEPC) for histidine residues and 1,2-epoxy-3-(p-nitrophenoxy)propane (EPNP) for aspartic acid residues. Aβ40 (1mg/ml) was incubated with 5 mM DEPC in 25 mM phosphate buffer (pH 7.4) for 2 h at 37 °C [24], and the formation of N-carbethoxy-histidine residues was followed by the characteristic increase in absorbance at 240 nm [25]. The sample was dialyzed to remove excess DEPC, and referred to as histidine-modified Aβ40: Aβ40 (H*). Aβ40 (2 μM) was incubated with 40 μM DFP in 1 ml water for 20 h at 37 °C, and after reaction it was adjusted to pH 7.4 with NaOH and the sample denoted serine-modified Aβ40 (S*). Similarly Aβ40 (2 μM) was incubated with 40 μM EPNP in 1 ml water for 20 h at 37 °C, yielding aspartic acid-modified Aβ40 (D*).

3.14. Inhibition of enzymatic activity by eight-residue peptides

Mixtures of 5 μM of each eight-residue peptide with Aβ42 (5 μM) were incubated at pH 7.4 and 37 °C for 60 min before reaction with casein substrate in EnzCheck. The enzymatic activity of the mixture was determined after 60 min incubation at 37 °C. As a control, the activity of Aβ42 without addition of any peptides was determined. The percent inhibition of Aβ42 activity by the short peptides was calculated as follows: percent inhibition=(1 − relative fluorescence intensity in the mixture of Aβ42 with short peptide/relative fluorescence intensity in Aβ42 control) × 100 (%).

4. Results

4.1. Cryptic epitopes of Aβ42

The reactivity of 6F/3D towards pH 4.6 treated-Aβ42 was greatly affected by increasing concentrations of the potent denaturant GdnHCl, whereas the sample incubated at pH 7.4 was unaffected. This finding suggests that GdnHCl induces unfolding of the sample when incubated at pH 4.6 (Fig. 2).

4.2. Screening eight-residue peptides that inhibit Aβ42 aggregation

We previously suggested that Aβ42 aggregates at pH 4.6 and that the aggregates exhibit PK resistance, whereas the Aβ conformer at pH 7.4 does not aggregate and remains sensitive
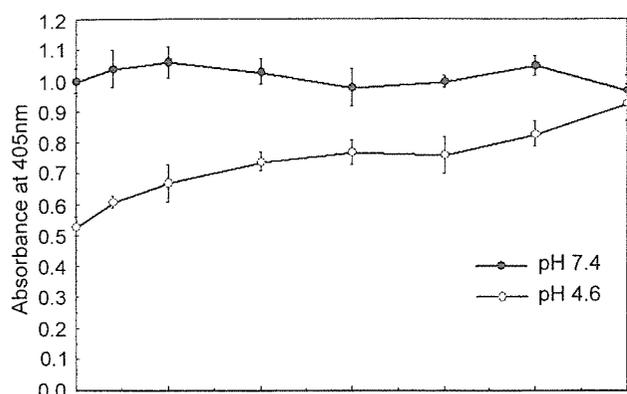
Fig. 2. GdnHCl-induced unfolding of pH-modified Aβ42. Samples of Aβ42 (4 μg/ml) incubated in pH 7.4 (●) and pH 4.6 (○) buffer were coated onto ELISA plates and treated with increasing amounts of GdnHCl prior to analysis by ELISA with 6F/3D antibody (1 μg/ml). Data are mean ± S.D. ($N = 6$).

to PK [12]. We evaluated the ability of short peptides to interfere with aggregation of Aβ42 at pH 4.6 by estimating the percentage of the protein remaining after PK digestion. After incubation at pH 4.6, in the absence of any short peptide, 33% of the Aβ42 protein remained, while only 16% of the protein remained after incubation at pH 7.4. Only three of the short peptides, Aβ15–22, 16–23 and 17–24 reduced the amount of protein remaining from that of the control at pH 4.6, to that of the control at pH 7.4; their values were 16%, 14% and 17%, respectively ($p < 0.001$; Fig. 3).

4.3. The active peptides also inhibit Aβ42 accumulation in glial cells

The inhibitory activity of the three short peptides on Aβ42 aggregation was confirmed by cell western dot blot

assay. The signal from the spot of Aβ42 at pH 4.6 was reduced by co-incubation with Aβ15–22 and Aβ16–23 (Fig. 4a). The average pixel density of each spot was analysed with NIH imaging software and the percentages of protein remaining after PK digestion were calculated from the spot density. Protein remaining was 10% in the Aβ42 control at pH 7.4 and 62% in the control at pH 4.6. The percentage protein remaining at pH 4.6 was reduced by addition of Aβ15–22 and Aβ16–23 (42% and 37%, respectively; Fig. 4b). Aβ16–23 was more active in this assay than peptide LPFFD (45%), a derivative of Aβ17–20, LVFF (data not shown).

4.4. Dose-dependence of Aβ42 activity on casein

Fig 5a presents the hydrolytic activity of Aβ42 at pH 7.4 over the range of 1–20 μM. The activity of Aβ42 (2.5 μM) showed 1.7 fluorescence intensity and similar reactivity was observed for 200 pM trypsin (data not shown).

4.5. Time-dependence of hydrolysis

The hydrolysis of casein at pH 7.4 by Aβ 42 and Aβ17–42 was examined as a function of time at 37 °C. The relative fluorescence intensity immediately increase to 0.88 upon addition of Aβ42 peptide, and eventually reached a plateau at 2.2 after 60 min. The maximum activity of Aβ17–42 was about 30% of Aβ42, over a similar time course (Fig. 5b).

4.6. The proteolytic activity of Aβ peptides depends on pH

The plateau fluorescence intensity reached in the casein hydrolysis assay was reduced to 0.53 at pH 4.6, a decrease
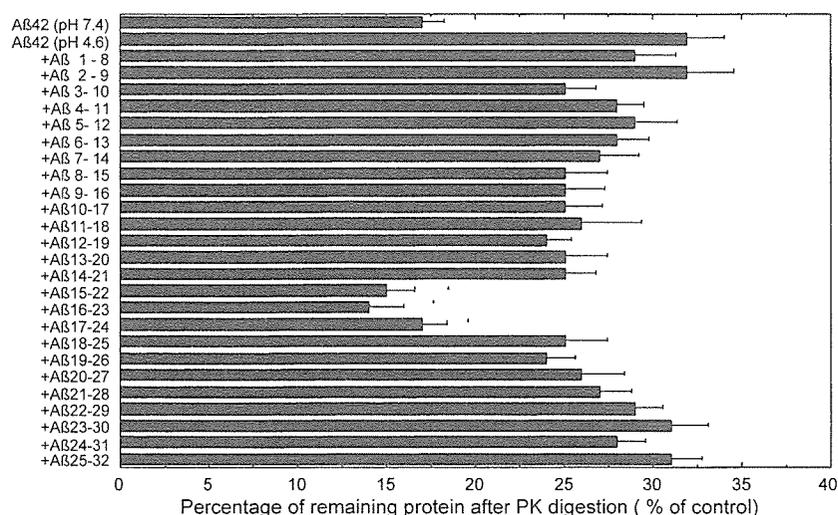
Fig. 3. Inhibition of acid-induced Aβ42 aggregation by Aβ fragments. Samples of Aβ42 (4 μg/ml) together with short peptides (4 μg/ml) at pH 4.6 were immobilized on the ELISA plate and, after digestion with PK at 10 μg/ml, the amount of protein remaining was determined with anti 5–10 antibody (1 μg/ml) by ELISA assay. The percentage of PK resistant protein in each sample was estimated as follows: (absorbance of remaining protein/initial protein) × 100 (%). Aβ42 (4 μg/ml) at pH 7.4 in the absence of any other peptide acted as control for PK sensitive Aβ42. Data are mean ± S.D. ($N = 6$).
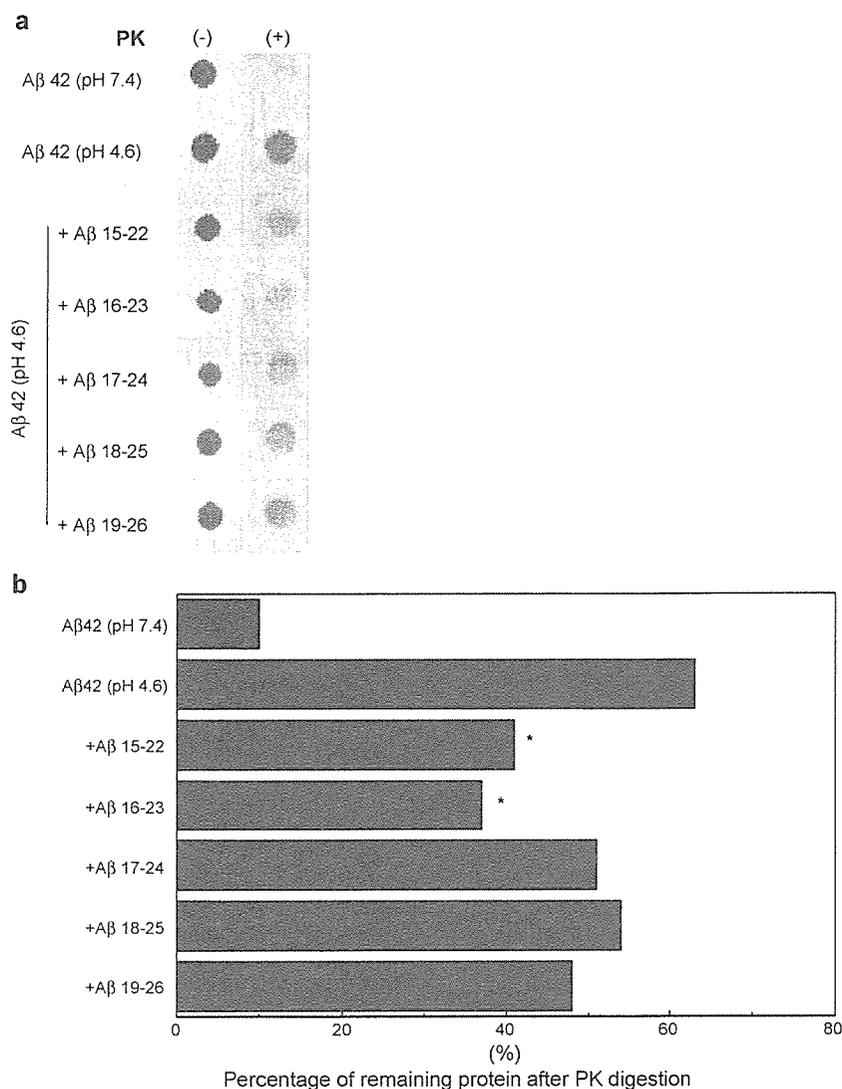
Fig. 4. Inhibition of Aβ42 accumulation in glial cells by short peptides. (a) Human glial cells (2 × 10⁴ cells/ml) were cultured for 7-days with pH 4.6-modified Aβ42 (4 μg/ml) in the presence or absence of the eight-residue peptides (4 μg/ml), Aβ15–22 to Aβ19–26 and the fraction of protein remaining after PK digestion (10 μg/ml) determined using the cell western dot blot system as described in Methods. Aβ42 at pH7.4 served as a control. (b) Quantification of PK resistant Aβ by computerized densitometry. The percentage of PK resistant protein was determined as follows: (pixel density of spot after PK digestion/density before digestion) × 100 (%).

of about 80%. At neutral pH, Aβ17–42 had approximately 40% of the activity of Aβ42, and this was almost unchanged by acid exposure. Aβ1–16 had no activity at either pH (Fig. 6).

4.7. Dependence of proteolytic activity on peptide length and effects of metal ions for the activity

It would appear that at neutral pH the hydrolytic activity of Aβ, and small peptides derived from it, depends on peptide length. The activities of Aβ40 and Aβ42 were 1.9 and 2.2 fluorescence intensity, respectively: Aβ17–42 had 45% of this activity, while Aβ1–16 and Aβ12–28 displayed no activity at all. The activity of Aβ1–28 was 0.4 fluorescence intensity corresponding to about 20% of Aβ40 activity

(Fig. 7a). The copper ion could inhibit the proteolytic activity of Aβ42 for casein by approximately 85% decrease and it was completely recovered with EDTA. Zinc and iron ions, however, had little affect on the activity (Fig. 7b).

4.8. Crucial amino acid residues for proteolytic activity

The activity of the Aβ40 derivatives in which glutamic acid is replaced by glutamine at position 11 or 22 was approximately 50% of Aβ40 itself. Chemical modification of the histidine residues at positions 6, 13 or 14, as well as modification of the serine residues at positions 8 and 26, almost completely abolished activity. Conversely, modification of the aspartic acid residues at positions 1, 7 or 23 had no effect (Fig. 8).
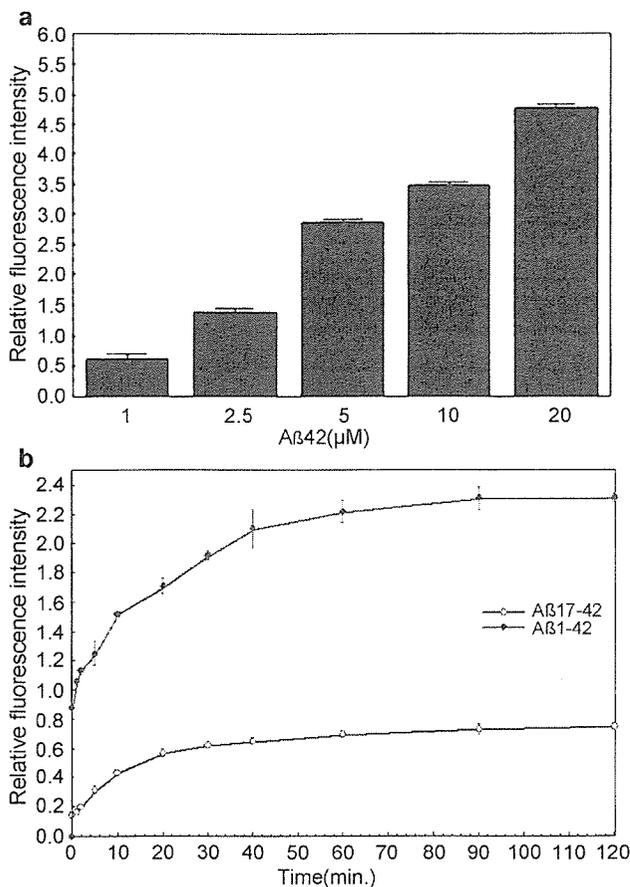
Fig. 5. (a) Dose-dependence of the proteolytic activity of Aβ42. The proteolytic activity of Aβ42 for casein at pH 7.4 was assayed using EnzCheck as described in Methods. The activity of the indicated concentrations of Aβ42 was determined after 1-h incubation at 37 °C. The relative fluorescence intensity was determined after subtraction of solvent fluorescence intensity. Data are mean ± S.D. (N = 3). (b) Time dependence of the proteolytic activities of Aβ42 and Aβ17–42. The activity of Aβ42 (●) and Aβ17–42 (○) at pH 7.4 for casein was assayed by EnzCheck as a function of incubation time. Data are mean ± S.D. (N = 6).

Fig. 7. (a) Dependence of the activity of Aβ peptides on peptide length. Aβ42, Aβ40, Aβ17–42, Aβ1–28, Aβ12–28 and Aβ1–16 at 5 μM were assayed for cleavage of casein at pH 7.4 using EnzCheck. Activity was determined after 1-h incubation at 37 °C. Data are mean ± S.D. (N = 6). (b) Effects of metal ions for the Aβ42 activity. $CuCl_2$, $ZnCl_2$ and $FeCl_2$ at 25 μM was incubated with Aβ42 (2.5 μM), respectively, at 37 °C for 1 h, and they were tested for enzymatic activity using EnzCheck. EDTA (50 μM) was also tested to affect the Cu^{2+} in the enzymatic activity. Data are mean ± S.D. (N = 6).

Fig. 6. pH-dependence of Aβ activity. Each of the Aβ-derived peptides of different length including Aβ42, Aβ17–42 and Aβ1–16 (5 μM) were assayed for proteolytic activity against casein using EnzCheck at pH 7.4 (□) and pH 4.6 (■). The activity was determined after 1-h incubation at 37 °C. Data are mean ± S.D. (N = 6).
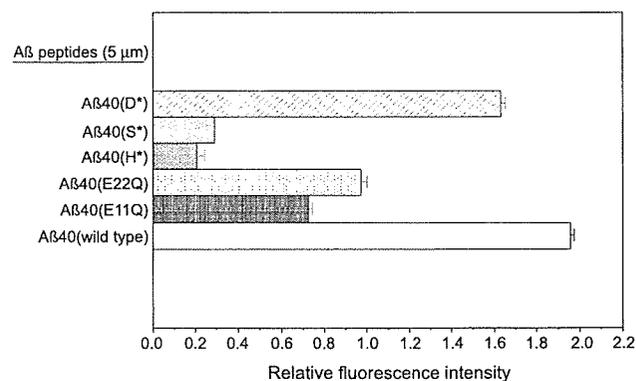

Fig. 8. Essential sequences and residues required for Aβ activity. Comparison of the proteolytic activities of Aβ40 (wild type), Aβ40 (E11Q), (E22Q), and chemically modified Aβ40; Aβ40 (*H), with histidine residues modified by DEPC; Aβ40 (*D), with aspartic acid modified by EPNP; Aβ40 (*S), with serine residues modified by DFP. Data are mean ± S.D. (N = 6).
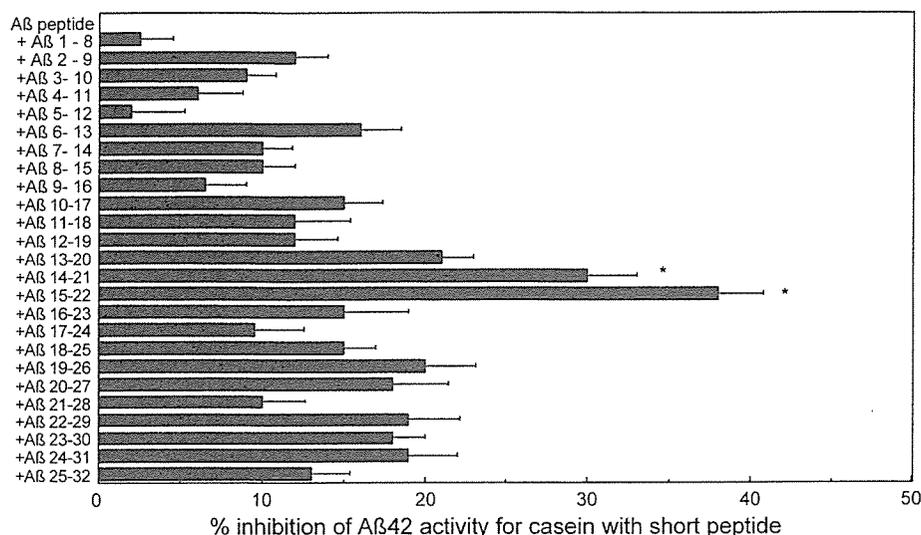
Fig. 9. Inhibition of Aβ42 activity by short peptides. Mixtures of each short peptide (5 μM) with Aβ42 (5 μM) were incubated at 37 °C and pH 7.4 for 60 min before assay with casein substrate. Proteolytic activity was determined after a further 60 min at 37 °C. As a control, the activity of Aβ42 without any short peptide was determined. Data are expressed as the percent inhibition as follows: percent inhibition=(1 − relative fluorescence intensity of mixture solution of Aβ42 and short peptide/that of Aβ42 without peptide) × 100 (%). Data are mean ± S.D. (N=6).

4.9. Inhibition of Aβ activity against casein by short peptides

The eight-residue peptides were tested for their ability to inhibit the hydrolytic activity of Aβ42 against casein. The percent inhibition was calculated as described in Methods. Only Aβ14–21 and Aβ15–22 were inhibitory, by about 30% and 38%, respectively (Fig. 9). Aβ15–22 also inhibited the hydrolytic activity of Aβ17–42 by about 45% (data not shown).

5. Discussion

Guanidine hydrochloride (GdnHCl) is a commonly used protein denaturant and at high concentrations unfolds the molten globule state. However, at low concentrations it can refold acid-unfolded proteins such as apomyoglobin and cytochrome c, stabilizing their molten globule state [26]. We have shown in the present study that it exposes amino acid residues 9–14 in Aβ42 (Figs. 1 and 2), supporting our previous observation that this region is hidden at pH 4.6. In addition GdnHCl caused unfolding of partially aggregated Aβ42, which may correspond to the intermediates that acquire proteinase resistance [12].

Studies of Aβ42 aggregation kinetics have suggested that aggregation of the hydrophobic form of Aβ42 can be selectively inhibited by the more soluble form, and that aggregation is driven by a hydrophobic effect of Aβ42 [27]. The central region of Aβ42 has been implicated in various biological functions including interaction with other proteins such as apolipoprotein E [3] and this region, comprising amino acid residues 19–25 has a very important influence on the aggregation and secondary structure of

Aβ peptide [28]. It has been suggested that residues 10–23 may provide the structural basis of the hydrophobic behaviour under physiological conditions [29]. Amino acid substitution studies indicate that the hydrophobic residues at position 17–20 are crucial for the amyloidogenic properties, with the very hydrophobic carboxy-terminal residues 29–42 corresponding to the transmembrane domain of Aβ42 [30,31] (Fig. 1). The five-residue peptide LPFFD, which is homologous to the central hydrophobic region 17–21 (LVFFA) of Aβ42, has been reported to inhibit Aβ fibrinogenesis in vitro, prevent neuronal cell death in culture and reduce Aβ deposition in the rat brain [32]. It has also been claimed that this peptide can reverse pre-existing Aβ fibrils [33]. Our pervious study of the pH-induced conformational transitions of Aβ42 also suggested that amino acids residues at positions 9–14 and 17–21 were responsible for the changes [12].

The ability of partial Aβ fragments around Aβ16–23 to inhibit Aβ42 aggregation was proved by both ELISA and cell western dot blot analysis (Figs. 3 and 4a,b). This may be due an ability to bind to the central hydrophobic region of Aβ42, including the pH-sensitive region, thereby destabilizing the interaction between Aβ monomers and/or oligomers necessary for fibril stability. As a result, the site of proteinase cleavage would be exposed, and it would become sensitive to proteinase K. Short fragments around Aβ16–23 may be produced in vivo in the normal processing steps, since α-secretase is reported to act between residues 16 and 17 [34], and cathepsin D in the lysosome acted in the region of residue 21 [35,36]. Moreover, exposure to the proteinase responsible for insulin degradation generates the Aβ17–24 fragment [37]. Hence, peptides around Aβ16–23 could be physiological products and may play an important role in inhibiting self-aggregation

of Aβ42 in vivo. In our present assay system Aβ16–23 inhibited Aβ42 aggregation better than the five-residue peptide LPFFD (data not shown). We have suggested that short peptides could facilitate the formation of mixed aggregates with Aβ42 that are sensitive to proteinase K and these findings raise the possibility that peptides around Aβ16–23 could be useful for treating amyloid plaque in the AD brain.

We have confirmed the proteolytic activity of Aβ42 for casein at neutral pH and have shown that Aβ17–42 also has some activity (Figs. 6 and 7a). pH shift from 7.4 to 4.6 decreased the activity of Aβ42 by about 76% and resulted in the same level of activity as Aβ17–42, which was almost unaffected by acid pH (Fig. 6). These data indicate that residues important for Aβ42 activity are pH sensitive and that these must be present in the region between residues 1 and 16. We speculate that residues around 9–14 and 17–21, which are affected by pH and induce conformational changes [12], may participate in the activity. Though Aβ42 requires the first 16 residues for full activity, Aβ1–16 itself has no activity (Figs. 6 and 7a). As there are no amino acid residues in Aβ29-42 capable of forming the active site, the region 1–28 must contain all of the amino acid residues essential for activity. Aβ1–28 corresponds to the extracellular domain and contains the first α-helix (Fig. 1), and had a low level of activity. It should be noted that although NMR studies of micelle-bound Aβ42 revealed the existence of an α-helix in this region [38], examination in water did not support that finding [39]. Despite the fact that the lengths of Aβ1–28 and Aβ17–42 are almost the same, Aβ17–42 showed higher activity than Aβ1–28 (Fig. 7a). We suggest that the region 29–42, which contains a second a-helix and corresponds to the transmembrane domain, is essential for full activity of Aβ42 and that serine 26 may contribute to the partial activity of Aβ17–42. Furthermore, residues 29–42 may be essential for stabilizing the first α-helix (residues 9–23), because these residues are reported to be essential for stabilizing the fibrils [40,41]. Although Aβ1–16 and Aβ12–28 may contribute residues crucial for Aβ42 activity, both are shorter than Aβ1–28 and lack the region required for stabilization. Metal ions including copper and zinc could induce conformational transition of Aβ42 at neutral pH and amino acids residues 9–14 (6F/3D epitope) participate to the changes [12] and in the present study, copper could also inhibit the enzymatic activity of Aβ42 (Fig. 7b). These results suggest that the 6F/3D region, which is associated with copper, might participate to the formation of the catalytic site of Aβ42.

Chemical modification of the serine, as well as the histidine residues of Aβ40, dramatically reduced proteolytic activity. However, modification of the aspartic residue has no affect (Fig. 8). Substitution of glutamic acid by glutamine at positions 11 and 22, in the putative α-helical region, also decreased activity by 50–60% (Fig. 8). The mutation at position 22 (E22Q) is known as the "Dutch type" and gives rise to familial early onset AD [42–44]. Such protein has

potent aggregative ability and neurotoxicity in PC12 cells (rat pheochromocytoma) [45]. Our previous data suggested that the glutamic acid at position 11 is very sensitive to acidic pH and is a key residue for preserving the conformation around region 9–21 [12]. The present findings suggest that serine and histidine residues participate directly in the proteolytic activity of Aβ42, indicating that this may be a serine proteinase-like activity. The glutamic acids at positions 11 and 22 may be required to preserve the conformation around the catalytic site and the 29–42 region may be essential to stabilize the conformer. Further experiments are required to identify the serine and histidine residues responsible for activity.

Interestingly, the proteolytic activity of Aβ42 was only inhibited by Aβ14–21 and Aβ15–22 (Fig. 9). Thus, Aβ15–22 can inhibit both Aβ42 aggregation at acidic pH and its serine proteinase-like activity at neutral pH, and we speculate that oligomerisation of Aβ42 may be required for full proteolytic activity. Aβ15–22: QKLVFFAE may be useful in the treatment of AD.

Acknowledgements

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特集　前頭前野機能の最前線—基礎から臨床まで

前 頭 葉 型 痴 呆 の 臨 床*

鉾 石 和 彦** 池 田　　学** 田 邉 敬 貴**

前頭葉型痴呆 (dementia of frontal lobe type：DFT) は 1988 年にマンチェスター (イギリス) のグループによって提唱された概念であり，その後，DFT に代わり前頭側頭型痴呆 (fronto-temporal dementia：FTD) という概念が提唱された。FTD は変性性痴呆では Alzheimer 病，Lewy 小体病に次いで多く，多彩な精神症状，行動異常を示すが，その多彩さが初期診断を困難にしている可能性がある。

はじめに

前頭葉型痴呆 (dementia of frontal lobe type：DFT) は 1988 年にマンチェスター (イギリス) のグループによって提唱された概念[28,34,46]であり，原発性変性性痴呆例のうち，前頭葉症状を主徴とする非 Alzheimer 型変性性痴呆疾患の総称である。その後，DFT に代わり，前頭側頭型痴呆 (fronto-temporal dementia：FTD)[47]という概念が提唱されたが，いまだに名称や診断基準をめぐる論議は続いている。ここでは FTD を中心に，その概念と臨床症状について述べる。

Ⅰ．歴史的事項

Pick は 1892 年から 1906 年にかけて，前頭葉および側頭葉の限局性脳萎縮と特異な精神神経症状を呈した一連の症例を報告し[32]，その後 Alzheimer による Pick 小体の発見[1]を経て，1926 年に Onari と Spatz により限局性大脳皮質萎縮の状態に対して Pick 病の名称が与えられた[31]。しかし，Pick 小体の有無の扱いをはじめ，臨床・病理学的な議論が続き，アルツハイマー病

(Alzheimer's disease：AD) のようなコンセンサスの得られた診断基準の確立には至らなかった。1980 年代になると画像診断技術の進歩により，著明な脳萎縮を示さないが，機能画像では脳の前方部の機能低下を示し，Pick 病と同様の臨床症状を呈する一群の症例が知られるようになり，イギリスのマンチェスターのグループが DFT という概念[28]を，スウェーデンのルンドのグループが frontal lobe degeneration of non-Alzheimer type (非アルツハイマー型前頭葉変性症：FLD) という概念[7]を同時期に独立して発表した。しかしこれらは，やや概念が曖昧であったこともあり，広く受け入れられることにはならなかった。そこで 1994 年に，両グループは共同で萎縮部位により忠実に FTD という臨床的ならびに神経病理学的な概念[49]を提唱し，診断基準を示した。これにより臨床症状と画像所見から，従来からの Pick 病にまつわる病理学的な論議を先送りし，脳の前方部，すなわち前頭葉を中心に原発性の病変を有する非アルツハイマー型の変性性痴呆疾患を，包括的に診断することが可能となった。

その字面から誤解されることもあるが，FTD はあく

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┌─────────────────────────────────────────────────────────┐
│   Manchester 1988              Lund 1987                 │
│                                                          │
│                                DAT                       │
│   DAT              ┌─ non Pick type ····· FLD            │
│   D non AT ──┬─ DFT ┼─ Pick type         Pick D          │
│              │      └─ MND type                          │
│              └─ others                   others          │
└─────────────────────────────────────────────────────────┘
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┌─────────────────────────────────────────────────────────┐
│        Lund and Manchester 1994                          │
│                                                          │
│   DAT                                                    │
│                    ┌─ FLD type                           │
│   FTD      ────────┼─ Pick type                          │
│                    └─ MND type                           │
│        others                                            │
└─────────────────────────────────────────────────────────┘
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┌─────────────────────────────────────────────────────────┐
│        Manchester 1996                                   │
│                                          ┌─ FLD type     │
│   DAT                         ┌─ FTD ────┼─ Pick type    │
│   fronto-temporal lobar       ┤  PA      └─ MND type     │
│   degeneration                └─ SD                      │
│        others                                            │
└─────────────────────────────────────────────────────────┘
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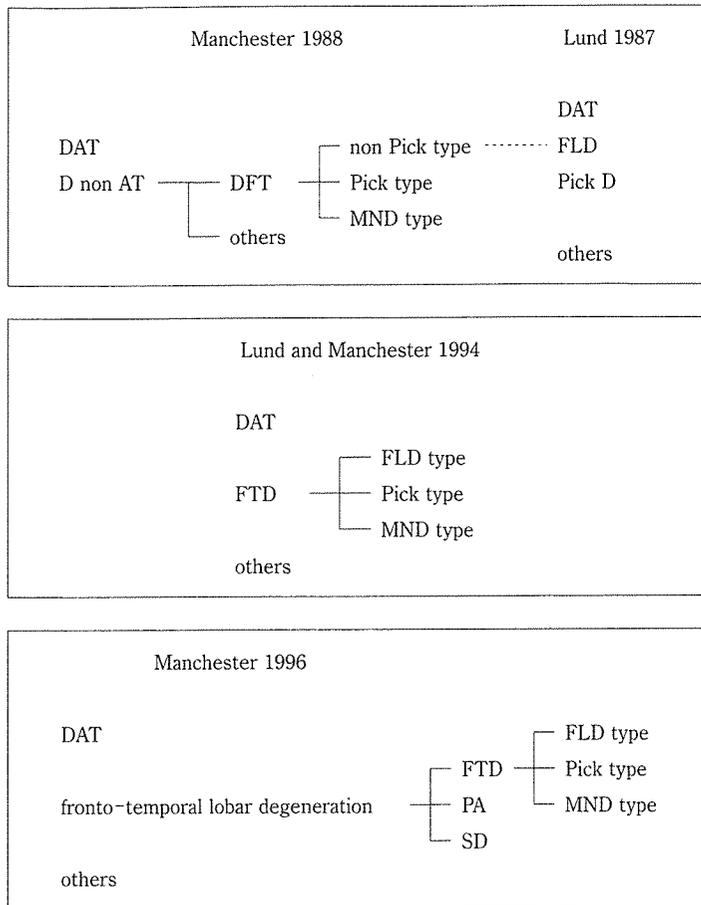**図 1　前頭葉型痴呆の概念の変遷**
DAT：dementia of Alzheimer type
D non AT：dementia of non Alzheimer type
DFT：dementia of frontal lobe type
FLD：frontal lobe degeneration of non-Alzheimer type
FTD：fronto-temporal dementia
FLD type：frontal lobe degeneration type
MND type：motor neuron disease type
PA：progressive non-fluent aphasia
SD：semantic dementia
(Hokoishi et al, 文献 12 より)

まで臨床的には前頭葉優位型 Pick 病に相当する概念であり，側頭葉優位の萎縮を呈し，失語症状を伴う側頭葉優位型 Pick 病を含む概念ではない。側頭葉優位の脳萎縮を呈する例については，FTD に先立って 1989 年にマンチェスターの Snowden らが語や物品の意味記憶 (semantic memory) の障害を呈し，従来，側頭葉優位型 Pick 病とされていた，語義失語を呈する変性性痴呆例に対して意味痴呆 (semantic dementia：SD) という概念を提唱している[41]。SD は 1992 年 Hodges ら[8] によって，その失語の特徴がより詳細に報告され，本邦では，従来，語義失語として知られていた症状の典型例が，この側頭葉優位の葉性萎縮例に認められることが明らかにされた[44]。しかし，それまで Pick 病の前頭葉優位型，側頭葉優位型として同一の疾患とみなされていたものが，FTD と SD という全く別の臨床症候群として取り扱われることによる混乱があり，1996 年にマンチェスターのグループは，前頭・側頭葉に原発性の病変を有する前頭側頭部脳変性症例に対し，前頭側頭葉変性症 (fronto-temporal lobar degeneration：FTLD) という概念を新たに提唱した[42]。そして，これを臨床症状から FTD, SD, そしてシルビウス裂周辺に病変を持ち，非流暢性の失語症状を呈する progressive non-fluent aphasia (PA), の 3 型に分ける新しい分類を提唱し，その背景となる病理所見についても記載した。すなわち FTLD と FTD が混同されていることが散見されるが，FTLD は FTD のほかに，SD や PA も含んださらに広い包括概念である (図 1)。

II. 前頭側頭葉変性症の疫学

FTLD は AD, Lewy 小体型痴呆 (dementia with Lewy bodies：DLB) に次いで 3 番目に多い変性性痴呆性疾患であるとされている[29]が，診断基準が変遷していることや初老期痴呆に対する疫学調査が極めて少ないため，これまでに FTD やそれを含む FTLD の頻度に関する詳細な報告は少ない。Neary は，FTD は初老期性痴呆の約 20% にみられた[30]と報告している。Ratna-

表 1 前頭側頭型痴呆（FTD）の臨床的診断特徴

性格の変化と社会的行動の障害（disordered social conduct）が，発症から疾患の経過を通して優位な特徴である。知覚，空間的能力，行為，記憶といった認知の道具的認知機能（instrumental function）は損なわれないか，比較的良好に保たれる。

I．主要診断特徴
- A．潜在的発症と緩徐な進行
- B．社会的対人行動（interpersonal conduct）の早期からの障害
- C．自己行動の統制（regulation of personal conduct）の早期からの障害
- D．早期からの情意（感情）鈍麻（emotional blunting）
- E．早期からの病識欠如

II．支持的診断特徴
- A．行動障害
  - 1．自己の衛生や身なりの障害
  - 2．精神の硬直化と柔軟性のなさ
  - 3．気の散りやすさ（distractibility）と根気のなさ（impersistence）
  - 4．口唇傾向と食餌嗜好の変化
  - 5．保続的行動と常同行動
  - 6．使用行動
- B．発話・言語
  - 1．発語量の変化（altered speech output）
    - a．自発話の低下と節約的発語　　　b．促迫発話（press of speech）
  - 2．常同言語
  - 3．反響言語
  - 4．保続
  - 5．緘黙症
- C．理学的徴候
  - 1．原始反射
  - 2．失禁
  - 3．寡動，固縮，振戦
  - 4．血圧の低下と不安定さ
- D．検査
  - 1．神経心理：前頭葉課題で有意な障害を示すが，顕著な記憶障害，失語，視空間性障害は伴わない
  - 2．脳波：臨床的に明らかな痴呆があるにもかかわらず通常の脳波は正常
  - 3．（形態的あるいは機能的，ないしその両方の）画像所見：前頭葉あるいは側頭葉前方部，ないしその両者の優位な異常

（Neary et al，文献 29 より）

valli らは初老期痴呆の地域住民における疫学的調査を行い，108 人の対象患者のうち AD が 25％，FTLD は 15.7％にみられたと報告している[33]。1997（平成 9）年の中山町における筆者らの調査[16]では，痴呆と診断された 65 歳以上の対象者のうち，AD 患者が 36％に対して FTLD 患者は 3％にすぎなかったが，初老期に発病した患者が他の地域の精神科病院などに転出している可能性があり，今後検討が必要である。他方，1996（平成 8）年 1 月から 2002（平成 14）年 12 月までの，筆者らの外来における連続症例では，330 名の痴呆患者のうち，臨床的に FTLD と診断された患者は 42 名

（12.7％）であった[18]。このうち FTD は 22 名（6％）であり，AD 患者は 215 名（65.1％），DLB 患者は 22 名（6.7％）であった。梶谷らは，九州大学医学部附属病院の，ものわすれ外来を受診した 75 名の患者について，AD 患者 33 名（45％）に対して FTD 患者は 5 例（7％）であり，DLB 痴呆患者は 2 例（3％）であったと報告している[19]。したがって外来患者では FTLD 患者は AD 患者と比較して稀な疾患とはいえず，初老期発症の痴呆症に限れば，その頻度はさらに高いと思われる。

表 2　前頭側頭葉変性症（FTLD）の一連の臨床症状（clinical syndrome）でよくみられる
　　　所見

Ⅲ．支持的特徴
　　A．65 歳以前の発症：一親等の親族に同症の家族歴
　　B．仮性球麻痺，筋力低下と筋萎縮，筋線維束れん縮（少数の患者で運動ニューロン疾患
　　　　が随伴する）
Ⅳ．除外診断的特徴
　　A．病歴と特徴（historical and clinical）
　　　　1．発作性のエピソードを伴う突然発症
　　　　2．発症と関連する頭部外傷
　　　　3．早期からの重篤な健忘
　　　　4．空間的見当識の障害
　　　　5．思考の脈絡を欠く語間代性促進性発話（logoclonic, festinant speech）
　　　　6．ミオクローヌス
　　　　7．皮質脊髄性の脱力（weakness）
　　　　8．小脳性失調
　　　　9．舞踏病アテトーシス
　　B．検査
　　　　1．画像所見：中心領域より後方優位の形態的ないし機能的障害，CT または MRI での
　　　　　　多巣性脳病巣
　　　　2．脳の代謝障害や炎症性疾患（多発性硬化症，梅毒，AIDS，ヘルペス脳炎など）を示
　　　　　　す所見
Ⅴ．相対的な除外診断的特徴
　　A．典型的な慢性アルコール中毒の病歴
　　B．持続性の高血圧
　　C．血管性疾患（狭心症，跛行など）の病歴

（Neary et al，文献 29 より）

Ⅲ．前頭側頭型痴呆の臨床的特徴[15]

　Neary らは 1998 年に FTLD の臨床診断基準[29]をあらためて示し，この中では，FTD の臨床診断基準も 1994 年のものと比較すると，整理されたものとなっている（表 1，2）。この臨床診断基準では，前頭葉優位型 Pick 病に認められる病識の欠如，人格変化などの臨床症状はほぼ包含され，AD との鑑別診断も十分可能なものとなっている。操作的診断基準にはなっているが，剖検例での裏付けはなされていない。FTD は病変が前頭葉から側頭葉前方部に限局し，それに由来する人格，行動変化をはじめとする前頭葉症候群を主徴とし，脳の後方部を中心にびまん性の脳萎縮を認め，記憶障害に加えて視空間性の障害や失行を呈する AD と対照的である[9]。例えば，診察場面では，一般的に AD 患者は愛想が良く，場あわせ応答や取り繕い反応[10,48]がみられるが，FTD の患者は無関心が強く，我が道を行く行動（going my way behavior）[47]があり，立ち去ってしまうこともある。以下，FTD の特徴的な臨床症状について述べる。

1．病識の欠如

　FTD では病識が病初期より欠如し，病感すら全く失われていると感じられることも多く，自己の能力低下に無関心であるように見える。病識は AD でも欠如するが質的に異なり，取り繕いがみられたり，自己の能力で遂行困難な事柄に直面すると，混乱し，不安，焦燥感が出現したりすることが多い。

2．感情・情動変化

　多幸的に変化していることが多いが，焦燥感が強く不機嫌を呈していることもあり，情意鈍麻や無表情もみられる。多幸的，児戯的な性格変化は，前頭葉眼窩面の障害が指摘されている[36]。異常な従順さ，柔和さがみられることもある。異常な従順さは，一般的には前頭葉損傷あるいは Klüver-Bucy 症候群のような側頭葉損傷でみられるが，側頭葉優位の萎縮を示す semantic dementia（SD）においてもしばしばみられ，神経解剖学的には扁桃体との関連が推測されている[45]。

3．脱抑制・反社会的行動

　欲求のおもむくままの，我が道を行く行動がみられ

る。これは，前方連合野から辺縁系への抑制が外れた結果と理解できる。ときに暴力行為がみられることはあるが，始終みられるわけではなく，常同行動が遮られたときや無理に集団作業療法に参加させようとしたときなどに出現しやすい。盗食や窃盗はしばしば認められるが，悪気はなく，指摘されてもあっけらかんとしている。脱抑制は，前頭葉眼窩面の障害で出現すると言われているが[36]，側頭葉との関連も指摘されている[50]。また，自閉症で注目されるようになった「心の理論 (a theory of mind)」は，他者の心を読んで，他者の行動に対応する社会知能に属する能力であると考えられている[2,35]。この社会知能に深く関わる脳部位は前頭葉眼窩皮質，上側頭回，ならびに扁桃体があげられる。FTD でもこれらの部位の障害と社会性の欠如がみられ，この理論からの神経心理学的ならびに神経放射線学的な検討が試みられている[6]。

4．自発性の低下

FTD では，短期間ではあるが後述の常同行動が出現し，その後自発性の低下が進行することが多い。若年発症例では自発性が急速に低下し，数年で無為無動となることもある。自発性の低下は脳血管性痴呆でもみられる症状の 1 つであるが，FTD の場合，病初期には常同行動や落ち着きのなさと共存してみられることが多く，昼寝をしているかと思うと常同的に周遊に出かけるといったように，瞬時に行動が切り換わる。声をかけないと 1 日中同じ場所でじっとしている，脳血管性痴呆の自発性の低下とはかなり様相が異なる。

また，一部自発性の低下と関連があると考えられる症状に「考え不精」がある。特に検査場面では少し複雑な課題になると，自ら考えようとはせず，検者にやらせようとしたり，よく考えずに即座に答えたりすることがしばしばみられ，後述する立ち去り行動へと続くことも多い。このため，正確に認知機能を評価することは難しい。

5．無関心

比較的初期からみられる。病棟でも他の患者に話しかけることはほとんど観察されない。考え不精や後述する立ち去り行動も無関心の関与が考えられる。通常，集団での活動やリハビリテーションにはなじまず，一人だけ寝そべったり，立ち去ったりすることもみられる。

6．常同行動[37]

しばしばみられるが，自発性の低下が目立ち始めると速やかに消失するため，見過ごされる可能性もある。神経基盤としては，側頭葉から前頭葉底面の病変および尾状核頭部との関連が重視される。病棟や施設では

デイルームの決まった椅子に座るという常同行動が形成されやすいが，日常生活では周徊（常同的周遊）や常同的食行動異常が目立つことが多い。これらは AD と鑑別の上で重要な症候であるといわれている[3,21,38]。言語面では，滞続言語の形で出現する。

1）周徊・常同的周遊 (roaming)

高頻度にみられる症状であり[23]，数 km の同じコースを毎日歩き続け，その途中，神社などの賽銭泥棒や花や果物を盗ってくるといった軽犯罪行為がみられる場合には，しばしば社会的な問題となる。とくに男性患者に出現することが多い。初診時，冬でも真っ黒に日焼けしていることもよくあり，夏には脱水症状で倒れるまで歩き続けることもあるので，注意を要する。進行期まで道に迷うことはなく，地誌的見当識障害を有する AD における徘徊とは対照的である。

2）常同的食行動異常[17]

決まった数少ない品目を毎日食べようとする。また，女性の場合は調理が常同的になり，同じ内容の食事を作ることが多い。2 年間も夫の弁当の内容が同一だった例もある。

3）時刻表的生活

常同行動が時間に強く規定された場合，時刻表的生活となり強迫性を帯びる。常同行動の時間的スパンは分・時間単位にとどまらず，日単位，週単位のこともあり，行動が曜日に規定されているような例もある。

4）反復行為

進行期に出現することが多く，絶えず膝を手で擦り続ける，手をパンパンと叩くといった行動がみられる。言語面では同語反復や反復書字の形で現れる。

5）強迫症状

比較的稀な症状である。症状自体は神経症でみられるものと同様であるが，自己の強迫症状に対する自我違和感が認められない点で異なる[43]。

7．被影響性の亢進 (Stimulus-bound behavior)

FTD でみられる被影響性の亢進ないし環境依存症候群[47]は，前方連合野が障害され後方連合野への抑制が外れ，後方連合野が本来有している状況依存性が解放された結果，すなわち外的刺激あるいは内的要求に対する被刺激閾値が低下し，その処理が短絡的で反射的，無反省となったものと理解できる[25]。日常生活場面では，介護者が首を傾げるのを見て同じように首を傾げる反響ないし模倣行為，何かの文句につられて即座に歌を歌い出す，他の患者への質問に先んじて応じる，視覚に入った看板などの文字をいちいち読み上げるといった行為で現れる。検査場面では物品や検者の動作が提示されたときに，反応しないように指示して
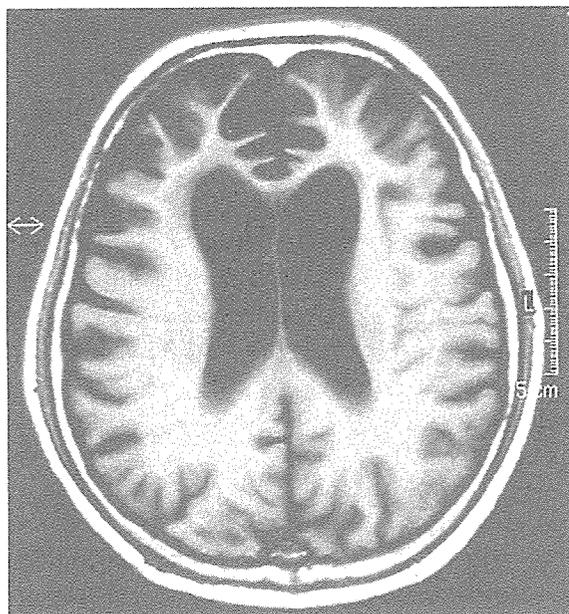
図 2　FTD（Pick 型）患者の頭部 MRI T1 強調像
前頭葉の著明な萎縮を認める。

も模倣したり，強迫的に言葉で応じてしまう強迫的言
語応答がみられる[40]。たとえば物品の場合は呼称し，
検者がチョキの形の手を見せたときは「チョキ」，「2」
などと言語化する。

8．転導性の亢進，維持困難

ある行為を持続して続けることができない，注意障
害，あるいは運動維持困難との関連が考えられる。必
ずしも外界の刺激に対して過剰に反応するだけではな
く，外界の刺激がなくても落ち着かない。立ち去り行
動は診察・検査場面でしばしば観察されるが，考え不
精や転導性の亢進とも関連のある症状のように思われ
る。

9．食行動の異常[17]

AD では食事をしたことを忘れ，食物の要求を繰り
返すことはあっても FTD のように，急激な体重増加
を伴うほどの大食は通常はみられない。FTD ではその
他にも甘い物，濃い味付けの物ばかりを好むといった
嗜好の変化がみられ，特定のメニューにこだわり，決
まった食物ばかりを食べようとする点（上述の常同的
食行動異常）でも AD と大きく異なる。病状の進行に伴
い，発動性の低下のため，全く食事をしない，食物を
飲み込まずに噛み続ける，食べ遊びをするといったこ
とがみられることもある。

IV．初期症状について

AD の場合，記憶障害が多くの場合初発症状であり，
かつ中核症状である。それでは，上記のように多彩な
臨床症状を呈する FTD にも，AD の記憶障害に相当す
るような初発症状は存在するのであろうか。最近，筆
者らは FTD の前駆症状と初発症状について，36 例の
FTD 患者と 17 例の SD 患者，そして 52 例の AD 患者
を比較検討した[38,39]。その結果，AD では初発症状の
6 割以上が記憶障害であったが，FTD では病初期から
社会行動の異常が前景に立ち，加えて患者一人当たり
の初発症状の数が多く，多彩であった。そしてこの症
状の多様性こそが FTD の特徴であり，同時に臨床診
断を困難にしている一因であると考えられた。

V．前頭葉変性症型について

Pick 病の病変部位は，AD が脳の後方優位であるの
に対して，前方優位である。AD の萎縮部位は病初期
には側頭葉内側部および頭頂葉に著明であり，側頭葉
外側および前頭葉へと進展する[4]。典型的な前頭葉優
位型 Pick 病ないし FTD の Pick 型では，頭部 CT，MRI
でナイフの刃状の脳萎縮像を前頭葉，側頭葉前方部に
認める（図 2）。詳細は本誌の前頭葉型痴呆の病理を参
照されたいが，FTD は病理学的には 3 つのサブタイプ
に分類されている。すなわちわが国でも多数の報
告[24,26]がなされている，神経症状を合併する運動
ニューロン型の他に，前頭葉と側頭葉に共通の病変分
布を有する 2 つの神経病理学的変化のタイプが含ま
れている。1 つは肉眼的な萎縮が軽度で，皮質の浅層
に軽度から中等度のグリオーシスを伴い，神経細胞の
脱落と海綿状変性がみられ，抗タウ，抗ユビキチン抗
体で検出される異常な構造物も存在せず，前頭葉変性
型（frontal lobe degeneration type：FLD 型）と診断され
るものである。もう 1 つは，皮質の全層にわたる高度
の線維性グリオーシス，神経細胞内封入体，腫大神経
細胞といった Pick 病の典型例の組織学的変化を有す
るものである。そして同程度の線維性グリオーシスを
呈するが，封入体や腫大神経細胞がみられない症例は，
Pick 型と分類した上で，より確定的な組織学的分類が
待たれるとされている。

FTD は上述のように病理組織学的な 3 つのサブタ
イプを含むとされながら，これらが 3 つの異なる疾患
単位であるかどうかという点は留保されている[42]。
FTD の臨床−神経病理学的対応では，神経症状を合併
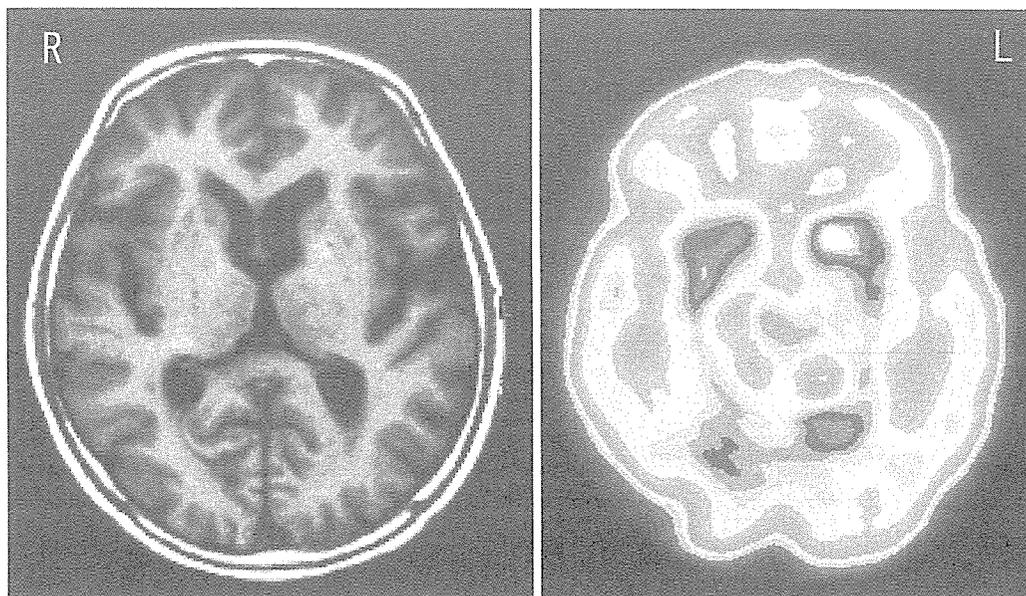する運動ニューロン型は別にして，FLD 型に関しては
Pick 型とは臨床症状による鑑別はできないという見

図 3

頭部 MRI では明らかな脳萎縮を認めないが，SPECT では脳の前方部の著明な血流低下を認める（文献 14 より）。

解が示されており，画像所見上，ナイフの刃状の萎縮などの特徴的な所見が認められないとされている以外は不明な点が多い。筆者らは画像所見上，脳の前方部に強い葉性萎縮を認め，脳の前方部の障害に由来する臨床症状を呈し，古典的な前頭葉優位型 Pick 病と考えられる症例と類似の臨床症状を呈し，SPECT にて脳の前方部の顕著な血流低下を認めながら，CT あるいは MRI では前頭葉あるいは側頭葉前方部に境界明瞭な，ナイフの刃状と称せられるような顕著な萎縮が認められない臨床例（図 3）を報告し[11]，本邦にも FLD 型が存在する可能性を示した。また，Kitagaki らは萎縮の程度の差異は白質の線維性グリオーシスの程度の差異を反映していると考え，MRI の T2 強調像とプロトン像の前頭葉白質の信号強度を検討した[20]。その結果，MRI 上で脳の前方部に境界明瞭ではなく，ナイフの刃状でもない軽度の萎縮を呈している FLD 型と考えられた症例では，白質の線維性グリオーシスが軽度であるため信号強度の変化が小さく，境界明瞭な限局性の萎縮を呈し，Pick 型と考えられる症例では白質の線維性グリオーシスが高度であるため信号強度の変化が大きく，この相違から両者の鑑別が可能であることを指摘している。病理学的特徴が臨床症状と無関係であるという点については，自験例による FLD 型と Pick 型の臨床症状の比較では，臨床症状に有意な差を認めず[12]，アメリカでも同様の報告がなされた[22]。しかし，

本邦では筆者らの報告を含めて FLD 型と思われる臨床報告[5,11,27]はあるものの，剖検例の報告はなく，Lund and Manchester group の FLD 型は，家族性の強い地域性疾患が含まれている可能性が高いという指摘[14]もあり，今後の症例の蓄積，特に剖検例の報告による欧米の症例との比較検討が期待される。

おわりに

分子生物学的には 1998 年に，frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) の原因遺伝子が発見され[13]，本邦でも兄弟例の報告[21]もされるなど，FTD は分子生物学的にも注目されてきている。しかし，DFT は，脳の前方部の障害を有し，独特の臨床症状を呈する症例群に与えられた名称であったが，その後 FTD では，病理学的な概念が取り入れられ，さらに分子生物学的な要素も加わった結果，その取り扱いに混乱を来した印象がある。また，最近注目されているタウオパチーという新しい概念では，tau 蛋白の異常の有無からグループ分けを行っている[34]。いずれにせよ，最終的な確定診断は病理解剖ないし分子生物学的研究に帰すると考えられるが，診察室を訪れる痴呆性疾患患者の，ケアを含めた対応に症候学は重要であり，また，FTD は前頭前野の機能を研究する上でも有用なモデルを提供していると思われる。