Amyloid β-protein (Aβ) associated with lipid molecules: immunoreactivity distinct from that of soluble Aβ

Katsuhiko Yanagisawa[a,*], JoAnne McLaurin[b], Makoto Michikawa[a], Avijit Chakrabartty[b], Yasuo Ihara[c]

[a] *Department of Dementia Research, National Institute for Longevity Sciences, 36-3 Gengo, Morioka, Obu 474, Japan*
[b] *Division of Molecular and Structural Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada*
[c] *Department of Neuropathology, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan*

Received 24 October 1997; revised version received 14 November 1997

Abstract We previously identified a novel amyloid β-protein (Aβ), that binds to GM1 ganglioside, in brains exhibiting the early pathological changes of AD. In this study, we raised monoclonal antibodies, using membrane fractions containing abundant GM1 ganglioside-bound Aβ as antigens. Monoclonal antibody 4396, produced in this study, immunoprecipitates Aβ42 in the membrane fractions of brains with diffuse plaques, but does not react with soluble Aβ42 or GM1 ganglioside. Furthermore, this antibody recognizes the Aβ bound to lipid vesicles containing GM1 ganglioside, and unexpectedly, phosphatidylinositol. In contrast, a control anti-Aβ monoclonal antibody does not recognize the Aβ bound to these lipid vesicles. These results indicate that Aβ associated with lipids has an immunoreactivity distinct from that of soluble A.

Key words: Alzheimer's disease; Amyloid β-protein; Monoclonal antibody; GM1 ganglioside

1. Introduction

The deposition of amyloid β-protein (Aβ), a proteolytic cleavage product of β-amyloid precursor protein (βAPP) [1], is an initial and invariable feature in the brains of subjects with Alzheimer's disease (AD). However, the molecular mechanism of this Aβ deposition remains unclear. To address this issue, we previously fractionated cerebral cortices from AD and Down's syndrome subjects, and healthy aged individuals using sucrose density gradient centrifugation, and searched for the initially deposited Aβ species [2]. In that study, we found a novel Aβ, with unique immunoreactivities, that tightly binds to GM1 ganglioside in the brains of subjects in the early stages of AD. Although Aβ is physiologically secreted into the extracellular space and GM1 ganglioside is expressed on the extracellular surface, particularly on synaptic membranes, we were unable to find this GM1 ganglioside-bound Aβ (GM1/Aβ) in the control cerebral cortices. Thus, we raised a possibility that GM1/Aβ is generated intracellularly due to an alteration in the membrane traffic or an abnormal trafficking of Aβ in AD brains. However, immunohistochemical studies, to date, have failed to demonstrate the intracellular deposition of Aβ in the brains of subjects with AD, which suggests that GM1/Aβ cannot be recognized by conventional antibodies raised against synthetic Aβ. The aim of this study is to determine whether Aβ associated with lipid molecules including GM1 ganglioside has an immunoreactivity distinct from those of soluble and amyloid Aβ. We raised monoclonal antibodies using membrane fractions containing GM1/Aβ as an antigen, and investigated those immunoreactivities to the Aβ in the membrane fractions from AD brains and to the Aβ bound to lipid vesicles.

*Corresponding author. Fax: +81 (562) 44-6594.
E-mail: katuhiko@nils.go.jp

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β-protein; βAPP, β-amyloid precursor protein; MG, mixed ganglioside; PC, phosphatidylcholine; PI, phosphatidylinositol

2. Materials and methods

2.1. Materials

The Aβ peptides (Aβ1-40, Aβ1-42) and GM1 ganglioside were obtained from Bachem California Inc. Phosphatidylcholine (PC) was obtained from Avanti Polar Lipids, and the mixed ganglioside (MG) fraction of bovine brain and phosphatidylinositol (PI) were obtained from Sigma. The antibodies used in this study were BC05 (specific for Aβ42/43), BAN052 (raised against Aβ1-16), whose characteristics have been described elsewhere [3,4], 4G8 (specific for Aβ17-24) (SENETECH) and an anti-human Aβ IgG (raised against Aβ8-17) (clone 6F/3c, Dako). Control mouse IgM was obtained from Zymed Laboratories, Inc.

2.2. Preparation of membrane fractions

To raise monoclonal antibodies against GM1/Aβ, membrane fractions were prepared from the cerebral cortices (800 mg) of subjects with abundant diffuse plaques using sucrose density gradient centrifugation as described previously [2]. To partially purify the GM1/Aβ, the membrane fractions, referred to as F1 in our previous paper [2], were solubilized in Laemmli sample buffer containing 8 M urea and then electrophoresed. Prepcell electrophoretic fractionation (Bio-Rad) was employed to obtain GM1/Aβ. Prepcell electrophoresis fractions were concentrated using the Microcon 3 concentrator (Amicon).

2.3. In vitro immunization and screening of hybridoma clones

In vitro immunization was performed as previously described [5,6]. Spleen cells from two mice (Balb/c, 6 weeks old, female) were suspended in 45 ml of Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 20% fetal calf serum (FCS), 50 μM β-mercaptoethanol, 20 μg/ml *N*-acetylmuramyl-L-alanyl-D-isoglutamine and plated onto a 24-well plate (0.45 ml/well, 4 wells). The cells were incubated with the antigen (1.2 ng Aβ/well) at 37°C and in 5% CO_2 for 4 days. PA-1 myeloma cells (3×10^6) (a gift from T. Arai) and the spleen cells (3×10^6) were gently mixed and placed in an electric fusion apparatus (Shimadzu) following washing with 0.3 M sucrose. These fused hybrid cells were then cultured in RPMI 1640 medium containing hypoxanthine-aminopterin-thymidine (HAT) in the feeder layer for 10 to 14 days. To obtain positive clones, the supernatants of hybridoma cell cultures were screened using the following two independent procedures. In the first procedure, 100 μl of the supernatants was applied to the smear of the membrane fractions from brains with abundant diffuse plaques, and immunoglobulins that bound to the smeared membrane fractions were visualized using a vectastain ABC-immunostaining kit (Vector Laboratories, Inc.). In the second procedure, blots of the same membrane fractions as used in the first

PII S0014-5793(97)01484-1

procedure, synthetic Aβ1-42 and purified GM1 ganglioside were immunoreacted with the supernatants. The blots were then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG or IgM. The bound enzyme activities were visualized using an enhanced chemiluminescence (ECL) detection system (Amersham, Buckinghamshire, UK). The clones showing positive reactivities both in the smear staining and in the immunoblotting of the membrane fractions, in conjunction with negative reactivities in the immunoblotting of synthetic Aβ1-42 and GM1 ganglioside, were selected. Subclass specific antisera were used to determine the immunoglobulin subtypes of the monoclonal antibodies.

2.4. Purification of IgM monoclonal antibody

Isolated hybrid cell clones, which had been determined to generate IgM monoclonal antibodies, were grown in the intraperitoneal space of mice. Ascites fluid containing monoclonal antibodies was obtained at 2–4 weeks after the injection of hybrid cell clones. The ascites fluid (14 ml) was filtered through a 0.45 μm membrane, dialyzed against 2 mM phosphate buffer (PB), pH 6.0 for 18 h at 4°C, and then centrifuged at 12 000 rpm for 20 min. The pellets were washed with PB and recentrifuged at 12 000 rpm for 20 min. The pellets were resuspended in PB and then dialyzed against 10 mM PB containing 0.15 M NaCl, pH 7.4. Monoclonal antibody 4396 is one of the purified IgM antibodies through this process.

2.5. Immunoprecipitation of Aβ in the membrane fraction

The cerebral cortices exhibiting abundant diffuse plaques were fractionated using sucrose density gradient centrifugation. The membrane fractions (protein; 250–400 μg) were sonicated in Tris-buffered saline containing 0.1% Tween 20 (TBS-T), pH 7.4 and immediately reacted with 50 μg of monoclonal antibody 4396, 50 μg of control IgM, 1 μg of 4G8, or 0.5 μg of BAN052 and incubated at 4°C overnight. The incubation mixtures were then centrifuged at 15 000 rpm for 15 min. The pellets were washed with ice-cold TBS-T and solubilized in RIPA buffer (50 mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholic acid and 1.0% Nonidet P-40) at 4°C for 30 min. The solubilized mixtures were then centrifuged at 15 000 rpm for 15 min and the supernatants were incubated with 20 μl of protein G Sepharose (PGS). In the case of incubation with IgM monoclonal antibody, PGS which had been precoated with goat IgG fraction against mouse IgM at 4°C for 60 min was used. Finally, the PGS was repeatedly washed with ice-cold Tris-buffered saline. For control immunoprecipitation, 1 μg of synthetic Aβ1-42 was suspended in RIPA buffer, and incubated with the same antibodies as used for the immunprecipitation of Aβ in the membrane fractions.

2.6. Western blotting

The PGS, following the incubation, was suspended in 25 μl of Laemmli sample buffer containing 8 M urea and boiled for 5 min. The solubilized antigen-antibody complexes were then applied to a 4–20% gradient Tris/tricine-SDS polyacrylamide gel (Multigel 4/20, Daiichi Pure Chemicals Co., Ltd.). Western blotting was carried out following the method of Ida et al. [7]. The separated proteins were blotted onto nitrocellulose membranes at 380 mA for 45 min using a semidry blotting apparatus. The blots were reacted with anti-Aβ monoclonal antibodies (BC05, BAN052) after boiling in phosphate-buffered saline (8.1 mM disodium hydrogen phosphate, 1.5 mM potassium dihydrogen phosphate, 137 mM sodium chloride and 2.7 mM potassium chloride, pH 7.4) to enhance immunoreactivity. Reactive Aβ on blots were visualized using the enhanced chemiluminescence (ECL) system (Amersham, Buckinghamshire, UK).

2.7. Fluorescein labeling of the monoclonal antibody

The monoclonal antibody 4396 and anti-human Aβ IgG (6F/3c) were labeled using 5(6) carboxyfluorescein-*N*-hydroxysuccinimide ester (Boehringer, Mannheim) according to the manufacturer's instructions. Fluorescently labeled antibodies were isolated by size exclusion chromatography on a G25 column.

2.8. Preparation of the lipid vesicles

Lipid vesicles were prepared as previously described [8]. Briefly, PC, PI and MG fractions were dissolved in chloroform/methanol (1:1, v/v). MG fractions were composed of predominantly GM1 ganglioside. All lipid aliquots were dried under a stream of nitrogen, lyophilized overnight and suspended in phosphate-buffered saline (PBS), pH 7.0. Lipid suspensions were subjected to 10 cycles of freeze-thawing in an acetone:dry ice bath. Small unilamellar vesicles were obtained by the sonication of the lipid suspensions for 20 min in a bath sonicator (Branson Ultrasonic Corp.). Pure gangliosides do not form stable vesicles and thus they were mixed with PC for vesicle formation.

2.9. Sucrose density gradient centrifugation

Samples contained 1000 μg of lipid vesicles (PC, MG/PC, PI) and ~2 μg of the fluorescein-labeled monoclonal antibody 4396, and, when present, the synthetic Aβ peptide (Aβ1-40 and Aβ1-42) content was 50 μg in PBS; the total volume of samples was 150 μl. Samples were layered onto a step gradient of 10–40% sucrose and centrifuged for 18 h at 35 000 rpm and 4°C. Following centrifugation, the samples were fractionated into 23 fractions. Each fraction was analyzed for fluorescein fluorescence and for phosphorous content using the Bartlett assay [9], to determine the presence of antibodies and lipid vesicles, respectively.

3. Results

3.1. Screening of monoclonal antibodies

Membranous structures (Fig. 1a) and a band with apparent molecular weight of 4 kDa (Fig. 1b) were immunoreacted with the media of positive clones. The two positive clones, obtained to date, were injected intraperitoneally using two Balb/c mice to produce antibody-rich ascites fluid. The class of antibodies, produced by both clones, was determined to be IgM using Western blotting. Monoclonal antibody 4396 was one of the purified IgM antibodies.

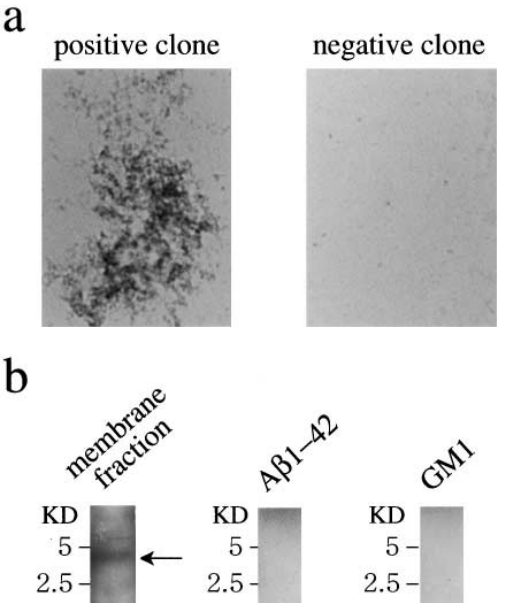

Fig. 1. Screening of hybridomas using smear immunostaining and Western blotting. a: Smear staining. Small aliquots of the membrane fractions of cerebral cortices with abundant diffuse plaques were smeared on glass slides and reacted with the supernatants of cultures of hybridomas. The bound immunoglobulins were visualized with the ABC-immunostaining kit. Membranous structures were reacted with the supernatants of positive hybridomas. Original magnification, 200×. b: Western blotting. The blots of the same membrane fractions as used in a. Synthetic Aβ42 and purified GM1 ganglioside were reacted with the supernatants of cultures of hybridomas. The blots were then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG or IgM. The bound enzyme activities were visualized by use of an enhanced chemiluminescence (ECL) detection system. The Aβs in the membrane fractions were recognized by IgM immunoglobulin in the supernatants of positive hybridomas (arrow). Note that neither synthetic Aβ1-42 nor purified GM1 ganglioside was recognized by the antibody.

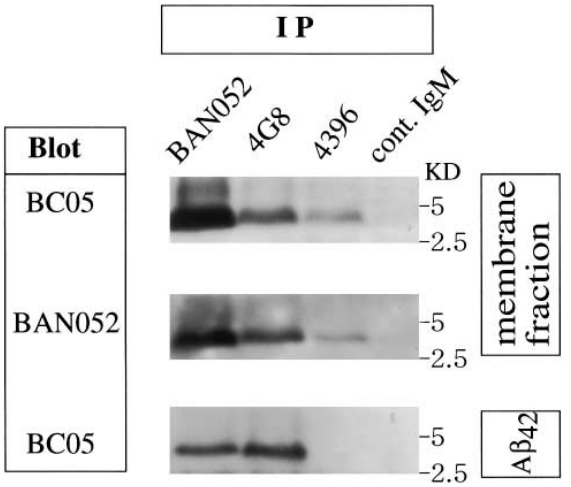

Fig. 2. Immunoprecipitation of Aβ in the membrane fractions and synthetic Aβ. Five hundred micrograms of the membrane fractions of cerebral cortices exhibiting abundant diffuse plaques and 1 μg of synthetic Aβ1-42 were incubated with 0.5 μg of BAN052, 1 μg of 4G8, 50 μg of monoclonal antibody 4396 and 50 μg of control IgM. The imunoglobulins were precipitated with protein G Sepharose directly or following precoating with goat anti-mouse IgM antiserum. The immunoprecipitates were blotted and reacted with BC05 or BAN052. Note that monoclonal antibody 4396 only reacts with Aβ in the membrane fractions.

3.2. *Immunoprecipitation of Aβ in the membrane fractions*

Aβ in the membrane fractions was readily immunoprecipitated with monoclonal antibody 4396 (Fig. 2). Aβ on the blots of the immunoprecipitates was recognized by BC05 and BAN052, which indicates that this Aβ species is Aβ1-42. This result agrees with our previous finding [2]. Monoclonal antibody 4396 does not immunoprecipitate synthetic Aβ1-42, whereas BAN052 and 4G8 immunoprecipitate Aβ in the membrane fraction as well as synthetic Aβ1-42 (Fig. 2). One possible explanation for the result shown in Fig. 2 is that BAN052 and 4G8 recognize the contaminating Aβ in the membrane fractions from soluble and/or amyloid core fractions. Control IgM does not immunoprecipitate Aβ in the membrane fractions or synthetic Aβ1-42 (data not shown). Thus, the present results indicate that the monoclonal antibody 4396 is a highly unique antibody that specifically recognizes Aβ in the membrane fractions.

3.3. *Recovery of fluorescein-labeled monoclonal antibody 4396 in sucrose density gradient centrifugation*

Preincubation of fluorescently labeled monoclonal 4396 with Aβ1-40/lipid vesicle or Aβ1-42/lipid vesicle were separated by sucrose density gradient centrifugation in a 10–40% discontinuous sucrose gradient to distinguish free and bound IgM. The reactivity of monoclonal antibody 4396 was found toward Aβ1-40 and Aβ1-42 which were bound to lipid vesicles containing GM1 ganglioside; however, these Aβ peptides were not recognized by control anti-Aβ antibody (Dako IgG) (Table 1). Furthermore, unexpectedly, Aβ peptides bound to PI vesicles were also recognized by monoclonal antibody 4396, while these Aβ peptides were again undetected by control anti-Aβ antibody (Table 1).

4. Discussion

Several lines of evidence indicate that the abnormal generation of Aβ due to altered processing of βAPP is a fundamental step in the initiation of AD. The cellular site(s) of Aβ generation, however, still remain(s) to be identified. Previous studies have revealed that Aβ is generated in acidic intracellular organelles such as endosomes following the internalization of βAPP [10,11], and it was recently reported that Aβ is generated in the endoplasmic reticulum [12–14]. Although Aβ has been detected in cell lysates following solubilization with detergents [12,14–19], previous immunocytochemical studies have failed to detect intracellular Aβ in situ. The reasons for this failure are assumed to be as follows. First, Aβ secretion may be so rapid that it cannot be easily detected. Second and more likely, Aβ, in or associated with intracellular organelles, may exhibit immunoreactivity distinct from that of soluble Aβ. In this context, it must be noted that the amino-terminal portion of Aβ exhibits various secondary structures including random coils, α-helices and β-sheets, depending on the solution conditions [20–25]: The amount of β-sheet structure increases in membrane-mimicking solvents [22]. Since most of the antibodies to Aβ have been raised against synthetic Aβ fragments, which are soluble in water, Aβ associated with the membrane lipids is most likely not detected using these conventional antibodies.

For the first time, our results clearly indicate that the Aβ associated with membrane lipids possesses immunoreactivity distinct from that of soluble Aβ. This is important to note when addressing the physiological intracellular trafficking of Aβ, or when considering the possibility of abnormal intracel-

Table 1
Relative extents of the binding of anti-Aβ antibodies to different vesicle preparations

	Percentage of fluorescein-labeled anti-Aβ antibody associated with vesicle[a]					
Anti-Aβ antibody	4396			Dako IgG		
vesicle type	vesicle alone	vesicle+ Aβ1-40	vesicle+ Aβ1-42	vesicle alone	vesicle+ Aβ1-40	vesicle+ Aβ1-42
PC	0	n.d.[b]	n.d.	0	n.d.	n.d.
MG/PC	0	2.1	1.9	0	0	0
PI	0.2	18.6	29.7	0	0	0

[a]Samples were layered onto a 10–40% sucrose gradient and centrifuged for 18 h at 35000 rpm and 4°C. After centrifugation, samples were fractionated into 23 fractions. Each fraction was analyzed for fluorescein fluorescence and for phospholipid using the Bartlett assay. The percentage of antibody associated with vesicle was determined using the following formula: % association = {(sum of fluorescence intensities of phospholipid-containing fractions)/(sum of fluorescence intensities of all fractions)} × 100(%).
[b]n.d., not determined. Aβ1-40 and Aβ1-42 do not bind to PC vesicles (McLaurin and Chakrabartty, unpublished data).
PC, phosphatidylcholine; MG, mixed ganglioside; PI, phosphatidylinositol.

lular accumulation of Aβ in the brains of subjects with AD. Monoclonal antibody 4396 was produced using membrane fractions of brains exhibiting the early pathological changes of AD, which were supposed to contain abundant GM1 ganglioside-bound Aβ1-42 [2]. However, in this study, the monoclonal antibody recognized both Aβ1-40 and Aβ1-42, which had been associated with MG/PC and PI vesicles. Thus, it is reasonable to conclude the following. First, a novel conformation that Aβ adopts via association with lipid vesicles is shared by Aβ1-40 and Aβ1-42. Second, GM1 ganglioside is most likely to be the lipid molecule which binds to Aβ in the brains of subjects with AD since GM1/Aβ was indeed detected in our previous study [2]; however, the conformational transition of Aβ caused by GM1 binding can be also induced via interaction with other lipid molecules. Currently, we have no explanation as to why monoclonal antibody 4396 exhibits greater binding affinity for Aβ bound to PI vesicles than for Aβ bound to MG/PC vesicles. In a previous study [26], it was reported that Aβ binds to acidic phospholipid via purely electrostatic interaction. Thus, this kind of interaction might cause a more dramatic alteration in the Aβ secondary structure than nonelectrostatic interaction between Aβ and ganglioside. In this context, it is noteworthy that PI is likely to be the most efficient inducer of β-structure in Aβ in phospholipids examined so far [27], although the specific secondary structure of Aβ recognized by monoclonal antibody 4396 remains to be determined. Recently, the binding of GM1 ganglioside to Aβ was confirmed in vitro [8,28,29]. Important points from these studies are (a) GM1/Aβ adopts a novel secondary structure as was determined by circular dichroism spectroscopy [8,28]; (b) GM1/Aβ causes membrane disruption [8] and (c) GM1/Aβ accelerates the rate of amyloid fibril formation [29]. The newly produced monoclonal antibodies in this study may be useful probes to gain new insight into the initial molecular mechanism of Aβ deposition, including the generation of GM1/Aβ, in the brains of subjects with AD.

Acknowledgements: The authors thank M. Yoshimura for the gift of autopsied human brains, R. Hosoda for assistance with the cell fusion and Y. Hanai for preparing this manuscript. J.M. is a recipient of a post-doctoral fellowship from the Alzheimer Society of Canada. This work was supported by a Research Grant for Longevity Sciences (8A-1) from the Ministry of Health and Welfare and by CREST (Core Research for Evolutional Science and Technology), Japan and by a grant from the Alzheimer's Association (USA) to A.C.

References

[1] Kang, J., Hemaire, H.G., Unter, A., Saldaum, J.M., Masters, C.L., Grzeschik, K.-H., Multhamp, G., Beyreuther, K. and Müller-Hill, B. (1987) Nature 325, 733–736.
[2] Yanagisawa, K., Odaka, A., Suzuki, N. and Ihara, Y. (1995) Nature Med. 1, 1062–1066.
[3] Suzuki, N., Iwatsubo, T., Odaka, A., Ishibashi, Y., Kitada, C. and Ihara, Y. (1994) Am. J. Pathol. 145, 452–460.
[4] Suzuki, N., Cheung, T.T., Cai, X.-D., Odaka, A., Otvos Jr., L., Eckman, C., Golde, T.E. and Younkin, S.G. (1994) Science 264, 1336–1340.
[5] Boss, B.D. (1984) Brain Res. 291, 193–196.
[6] Gilbert, P., Mundel, P. and Minuth, W.W. (1990) J. Histochem. Cytochem. 38, 1919–1925.
[7] Ida, N., Hartman, T., Pantel, J., Schroder, J., Zerfass .F, R., Forstl, H., Sandbrink, R., Masters, C.L. and Beyreuther, K. (1996) J. Biol. Chem. 271, 22908–22914.
[8] McLaurin, J. and Chakrabartty, A. (1996) J. Biol. Chem. 271, 26482–26489.
[9] Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
[10] Haass, C., Koo, E., Mellon, A., Hung, A.Y. and Selkoe, D.J. (1992) Nature 357, 500–503.
[11] Koo, E.H. and Squazzo, S. (1994) J. Biol. Chem. 269, 17386–17389.
[12] Wild-Bode, C., Yamazaki, T., Capell, A., Leimer, U., Steiner, H., Ihara, Y. and Haass, C. (1997) J. Biol. Chem. 272, 16085–16088.
[13] Hartmann, T., Bieger, S.C., Brühl, B., Tienari, P.J., Ida, N., Allsop, D., Roberts, G.W., Masters, C.L., Dotti, C.G., Unsicker, K. and Beyreuther, K. (1997) Nature Med. 3, 1016–1020.
[14] Cook, D.G., Forman, M.S., Sung, J.C., Leight, S., Kolson, D.L., Iwatubo, T., Lee, V.M.-Y. and Doms, R.W. (1997) Nature Med. 3, 1021–1023.
[15] Wertkin, A.M., Turner, R.S., Pleasure, S.J., Golde, T.E., Younkin, S.G., Trojanowski, J.Q. and Lee, V.M.-Y. (1993) Proc. Natl. Acad. Sci. USA 90, 9513–9517.
[16] Fuller, S.J., Storey, E., Li, Q.-X., Smith, A.I., Beyreuther, K. and Masters, C.L. (1995) Biochemistry 34, 8091–8098.
[17] Turner, R.S., Suzuki, N., Chyung, A.S.C., Younkin, S.G. and Lee, V.M.-Y. (1996) J. Biol. Chem. 271, 8966–8970.
[18] Martin, B.L., Schrader-Fischer, G., Busciglio, J., Duke, M., Paganetti, P. and Yankner, B.A. (1995) J. Biol. Chem. 270, 26727–26730.
[19] Tienari, P.J., Ida, N., Ikonen, E., Simons, M., Weidemann, A., Multhaup, G., Masters, C.L., Dotti, C.G. and Beyreuther, K. (1997) Proc. Natl. Acad. Sci. USA 94, 4125–4130.
[20] Hollosi, M., Otvos Jr., L., Kajtar, J., Percel, A. and Lee, V.M.-Y. (1989) Peptide Res. 2, 109–113.
[21] Barrow, C.J. and Zagorski, M.G. (1991) Science 253, 179–182.
[22] Otvos Jr., L., Szendrei, G.I., Lee, V.M.-Y. and Mantsch, H.H. (1993) Eur. J. Biochem. 211, 249–257.
[23] Fabian, H., Szendrei, G.I., Mantsch, H.H. and Otvos Jr., L. (1993) Biochem. Biophys. Res. Commun. 191, 232–239.
[24] Talafous, J., Marcinowski, K.J., Klopman, G. and Zagorski, M.G. (1994) Biochemistry 33, 7788–7796.
[25] Kirshenbaum, K. and Daggett, V. (1995) Biochemistry 34, 7629–7639.
[26] Terzi, E., Holzemann, G. and Seeling, J. (1995) J. Mol. Biol. 252, 633–642.
[27] McLaurin, J. and Chakrabartty, A. (1997) Eur. J. Biochem. 245, 355–363.
[28] Choo-Smith, L.-P. and Surewicz, W.K. (1997) FEBS Lett. 402, 95–98.
[29] Choo-Smith, L.-P., Garzon-Rodriguez, W., Glabe, C.G. and Surewicz, W.K. (1997) J. Biol. Chem. 272, 22987–22990.