A β-amyloid peptide variant related with familial Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis is poorly eliminated by cathepsin D

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Abstract The cerebral deposition of 40–42 residue amyloid β-protein (Aβ) is a characteristic of Alzheimer's disease. Cathepsin D is possibly involved in the intracellular clearance of Aβ (Hamazaki, H. (1996) FEBS Lett., in press). The present work shows that cathepsin D hydrolyzes wild-type Aβ 20 times faster than a variant Aβ with a substitution at residue 21 from Ala to Gly. Since the substitution has been linked to familial Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis (Hendriks et al. (1992) Nature Genet. 1, 218–221), the present observations suggest that the inefficient elimination of Aβ by cathepsin D is capable of being one of causes of the amyloid fibril formation.

Key words: Alzheimer's disease; Amyloidosis; β-Amyloid; Cathepsin D

1. Introduction

The cerebral deposition of amyloid β-peptide (Aβ) is one of the hallmarks of Alzheimer's disease [1,2]. The 40–42 residue Aβ is normally produced by proteolytic processing from β-amyloid precursor protein (βAPP), a 110–130 kDa type 1 integral membrane glycoprotein. Aβ is secreted from many cells including neurons [3,4] but the overproduction of Aβ or the production of more aggregatable Aβ variants are likely to lead to the formation of neuritic plaques and vascular deposits in the brain microvessels [3].

The missense mutation at codon 692 in βAPP_{770} from Ala to Gly has been linked to either hereditary cerebral hemorrhage with amyloidosis (HCHWA) or presenile dementia similar to familial Alzheimer's disease (FAD) [5]. This mutation leads to generation of a variant Aβ, Aβ(Ala21Gly). Recently, it has been shown that this mutation causes increased production of Aβ in vitro, in which human kidney 293 cells that were stably transfected to express βAPP(Ala692Gly) produced increases in secretion of Aβ and decreases in secretion of 3-kDa Aβ-related peptides with Val^{18} and Phe^{20} at their NH_2-termini [6]. One possible cause of the excessive secretion of Aβ in this mutant is supposed to be the decease in the intracellular degradation of the variant Aβ, although the responsible proteinase(s) have not been identified.

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Abbreviations: Aβ, amyloid β-peptide; βAPP, β-amyloid precursor protein; FAD, familial Alzheimer's disease; HCHWA, hereditary cerebral hemorrhage with amyloidosis; HCHWA-D, hereditary cerebral hemorrhage with amyloidosis of the Dutch type; RP-HPLC, reverse-phase high-performance liquid chromatography; TFA, trifluoroacetic acid

In the previous paper, we have shown that cathepsin D is a major proteinase in the brain that is able to eliminate full-length Aβ by hydrolyzing specifically between Phe^{19} and Phe^{20} [7]. The present work shows that cathepsin D hydrolyzes wild-type Aβ over 20 times faster than a variant Aβ related with HCHWA/FAD. The results suggest that inefficient degradation of the variant Aβ by cathepsin D can be a cause of excessive generation of the amyloidogenic peptide in the case of HCHWA/FAD.

2. Materials and methods

$Aβ_{1-28}$ was purchased from American Peptide Co. (Sunnyvale, CA). $Aβ_{1-28}$(Ala21Gly) was synthesized by Biosynthesis Inc. (Lewisville, TX) and purified on RP-HPLC in our laboratory. $Aβ_{12-28}$, $Aβ_{12-28}$(Ala21Gly), and $Aβ_{12-28}$(Glu22Gln) were prepared using Fmoc-chemistry in our laboratory and purified on RP-HPLC. The purity of all synthetic peptides were above 98% on RP-HPLC. Cathepsin D was purified from rat brain as described previously [7].

Aβ was hydrolyzed at a substrate concentration of 50 μM with 0.12 μg of cathepsin D in 20 μl of 0.1 M acetate buffer, pH 4.5, at 37°C. Hydrolyzed products were analyzed on RP-HPLC using an Inertsil 300C8 column (4.6×100 mm, GL Science, Japan). Column was eluted with a 20 min-linear gradient of 0–40% acetonitrile in 0.05% TFA at a flow rate of 1 ml/min and fractions with UV absorbance at 215 nm were collected for peptide sequencing. Peptides were sequenced on an Applied Biosystems model-491 protein sequencing system.

3. Results

In this study, synthetic peptides of Aβ which include Phe^{19}–Phe^{20} but are shorter than the full-length Aβ, $Aβ_{1-42}$, were used as substrate to compare susceptibility to cathepsin D, since cathepsin D specifically cleaves Aβ between Phe^{19}–Phe^{20} and the full-length Aβ is not suitable for RP-HPLC analysis due to low recovery from the column (Fig. 1). $Aβ_{12-28}$ and $Aβ_{1-28}$ have partial sequences of the wild-type Aβ. $Aβ_{12-28}$(Ala21Gly) and $Aβ_{1-28}$(Ala21Gly) have an amino acid substitution related with HCHWA/FAD [5], and $Aβ_{12-28}$(Glu22Gln) has a sequence related with hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) [8].

In the previous work it has been shown that cathepsin D is able to hydrolyze full-length Aβ specifically between Phe^{19} and Phe^{20} producing less-aggregatable fragments [7]. Since amino acid substitution at P2 or P3 position may affect the cleavage by cathepsin D, $Aβ_{12-28}$, $Aβ_{12-28}$(Ala21Gly), and $Aβ_{12-28}$(Glu22Gln) were incubated with cathepsin D and subsequently hydrolyzed products were separated on RP-HPLC and sequenced (Fig. 2). Cathepsin D hydrolyzed all the substrates commonly between Phe^{19} and Phe^{20}, and no other cleavages were observed under the experimental conditions.

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	1 ↓ 28
$A\beta_{1-28}$	DAEFRHDSGYEVHHQKLVFFAEDVGSNK
$A\beta_{12-28}$	VHHQKLVFFAEDVGSNK
$A\beta_{1-28}$(Ala21Gly)	DAEFRHDSGYEVHHQKLVFF**G**EDVGSNK
$A\beta_{12-28}$(Ala21Gly)	VHHQKLVFF**G**EDVGSNK
$A\beta_{12-28}$(Glu22Gln)	VHHQKLVFFA**Q**DVGSNK

Fig. 1. Structure of Aβ used as substrate in this work. Substituted amino acids in Aβ variants related with HCHWA/FAD and HCHWA-D were highlighted. Arrow indicates the cleavage site by cathepsin D.

However, the hydrolysis rate was very different; half of $A\beta_{12-28}$ was hydrolyzed within 5 min, whereas it took over 100 min to degrade half of $A\beta_{12-28}$(Ala21Gly) under the experimental conditions (Fig. 3). In contrast to $A\beta_{12-28}$(Ala21Gly), the hydrolysis rate of $A\beta_{12-28}$(Glu22Gln) was almost identical with that of $A\beta_{12-28}$. The effects of substitution at residue-21 on the susceptibility to cathepsin D were also confirmed using longer peptides, $A\beta_{1-28}$ and

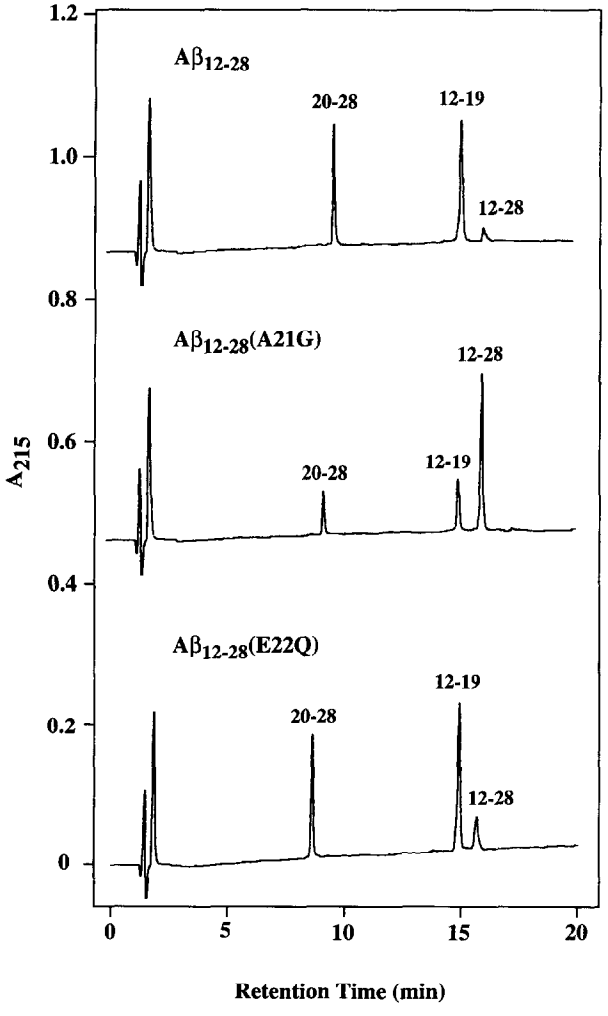

Fig. 2. Hydrolysis of $A\beta_{12-28}$, $A\beta_{12-28}$(Ala21Gly), and $A\beta_{12-28}$-(Glu22Gln) by cathepsin D. Substrate (1 nmol) was incubated with 0.12 μg of cathepsin D. Incubation times of $A\beta_{12-28}$, $A\beta_{12-28}$(Ala21Gly), and $A\beta_{12-28}$(Glu22Gln) were 15 min, 150 min, and 15 min, respectively.

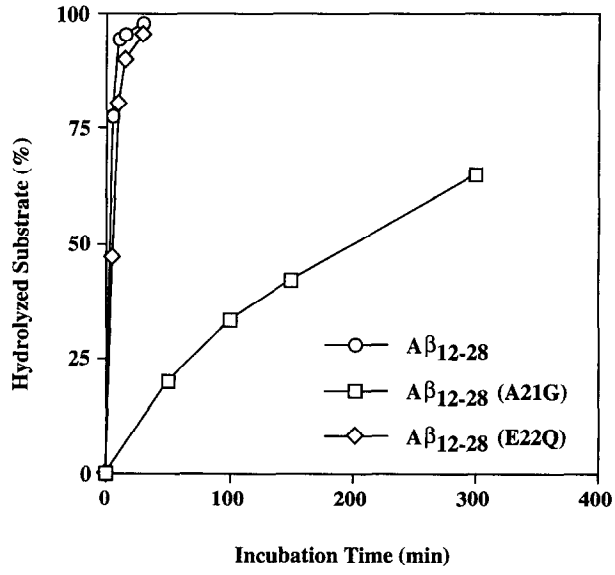

Fig. 3. Time course of hydrolysis of $A\beta_{12-28}$ and the variants by cathepsin D. Substrate (1 nmol) was incubated with 0.12 μg of cathepsin D in 0.1 M acetate buffer, pH 4.5.

$A\beta_{12-28}$(Ala21Gly) (Fig. 4). The data indicate that the substitution at residue-21 from Ala to Gly made Aβ less susceptible to proteolysis by cathepsin D.

4. Discussion

The present data indicate that (1) cathepsin D hydrolyzes unsubstituted Aβ, Aβ(Ala21Gly), and Aβ(Glu22Gln) between Phe^{19} and Phe^{20} and (2) Aβ(Ala21Gly) is poorly hydrolyzed by cathepsin D as compared with unsubstituted Aβ and Aβ-(Glu22Gln).

To date, six missense mutations of βAPP have been linked to cerebral hemorrhage with amyloidosis and Alzheimer's disease. Among them, two mutations lead to amino acid substitution within Aβ region. One is the mutation at codon 692 from Ala to Gly in βAPP_{770}, which generates Aβ(Ala21Gly) and is linked to HCHWA/FAD [5]. The other is the mutation at codon 693 from Glu to Gln in βAPP_{770}, which generates Aβ(Glu22Gln) and is linked to HCHWA-D [8]. Recently, it has been shown that cultured cells that were stably transfected to express βAPP(Ala692Gly) produced relative increases in secretion of Aβ and relative decreases in secretion of 3-kDa Aβ fragments [6]. Therefore, it seems plausible to suppose that the mutation linked to HCHWA/FAD produces the variant Aβ that is resistant to the intracellular clearance. In the case of HCHWA-D, the expression of the mutant βAPP in several lines of cells did not affect the amount of secreted Aβ [9–11]. The amyloid fibril formation in HCHWA-D may be attributed to the nature of the variant Aβ rather than to the amounts of secreted Aβ, since it has been demonstrated that the Aβ with a substitution at residue-22 from Glu to Gln is much easier to form amyloid fibril than the unsubstituted one [12]. Supposing that cathepsin D is involved in the intracellular clearance of Aβ, the results presented here are consistent with the observations in cultured cells [8–11].

It has been shown that the human kidney 293 cultured cells double-transfected to overexpress both human βAPP and cathepsin D secreted much less Aβ than the control cells trans-

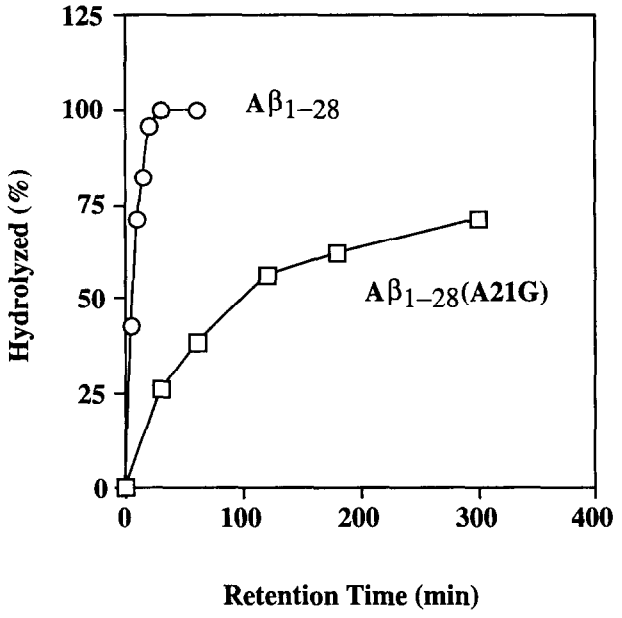

Fig. 4. Time course of hydrolysis of $A\beta_{1-28}$ and $A\beta_{1-28}$(Ala21Gly) by cathepsin D. Substrate (1 nmol) was incubated with 0.12 μg of cathepsin D in 0.1 M acetate buffer, pH 4.5.

fected to overexpress only human βAPP [13], suggesting that cathepsin D is involved in reducing the generation of Aβ in vivo. Although it has been described that cathepsin D was capable of producing the cleavages resulting in Aβ generation, in which relatively short peptides were used as substrate [14,15], the previous work [7] and the studies using cultured cells [13] indicate that cathepsin D also contributes to reduction of the aggregatable peptide. In conclusion, I propose that the substitution at codon 692 (Gly for Ala) in βAPP_{770} leads to produce a variant Aβ that is resistant to the clearance by cathepsin D, which results in providing more precursors for aggregation.

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References

[1] Glenner, G.G. and Wong, C.W. (1984) Biochem. Biophys. Res. Comm. 120, 885–890.
[2] Masters, C.L., Simons, G., Weinman, N.A., Multhaup, G., McDonald, B.L. and Beyreuther, K. (1985) Proc. Natl. Acad. Sci. USA 82, 4245–4249.
[3] Selkoe, D.J. (1994) Ann. Rev. Cell Biol. 10, 373–403.
[4] Checler, F. (1995) J. Neurochem. 65, 1431–1444.
[5] Hendriks, L., van Duijn, C.M., Cras, P., Cruts, M., Van Hul, W., van Harskamp, F., Warren, A., McInnis, M.G., Antonarakis, S.E., Martin, J.-J., Hofman, A. and Van Broeckhoven, C. (1992) Nature Genet. 1, 218–221.
[6] Haass, C., Hung, A.Y., Selkoe, D.J. and Teplow, D.B. (1994) J. Biol. Chem. 269, 17741–17748.
[7] Hamazaki, H. (1996) FEBS Lett. (in press).
[8] Levy, E., Carman, M.D., Fernandez-Madrid, I.J., Power, M.D., Lieberburg, I., van Duinen, S.G., Bots, G.T.A.M., Luyendijk, W. and Frangione, B. (1990) Science 248, 1124–1126.
[9] Felsenstein, K.M. and Lewis-Higgins, L. (1993) Neurosci. Lett. 152, 185–189.
[10] Maruyama, K., Usami, M., Yamao-Harigaya, W., Tagawa, K. and Ishiura, S. (1991) Neurosci. Lett. 132, 97–100.
[11] Sahasrabudhe, S.R., Spruyt, M.A., Muenkel, H.A., Blume, A.J., Vitek, M.P. and Jacobsen, J.S. (1992) J. Biol. Chem. 267, 25602–25608.
[12] Wisniewski, T., Ghiso, J. and Frangione, B. (1991) Biochem. Biophys. Res. Comm. 179, 1247–1254.
[13] Munger, J.S., Haass, C., Lemere, C.A., Shi, G.-P., Wong, W.S.F., Teplow, D.B., Selkoe, D.J. and Chapman, H.A. (1995) Biochem. J. 311, 299–305.
[14] Ladror, U.S., Snyder, S.W., Wang, G.T., Holzman, T.F. and Krafft, G.A. (1994) J. Biol. Chem. 269, 18422–18428.
[15] Evin, G., Cappai, R., Li, Q.-X., Culvenor, J.G., Small, D.H., Beyreuther, K. and Masters, C.L. (1995) Biochemistry 34, 14185–14192.