

Aluminum inhibits proteolytic degradation of amyloid β peptide by cathepsin D: A potential link between aluminum accumulation and neuritic plaque deposition

Takashi Sakamoto^a, Hisaka Saito^a, Kazuyuki Ishii^a, Hidenobu Takahashi^a,
Shinzo Tanabe^b, Yuki Ogasawara^{a,*}

^a Department of Environmental Biology, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

^b Department of Analytical Biochemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

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Abstract Neuritic plaques are the key pathological feature of Alzheimer's disease, and amyloid β (A β) peptides are major component of these plaques. In this study, we demonstrated the influence of aluminum (Al) on the A β peptide degradation by cathepsin D. Al did not directly affect the cathepsin D activity using small synthetic substrate. However, when A β peptides were used as substrate, the apparent inhibitory effect of Al on cathepsin D activity was observed. This inhibitory effect disappeared by treatment of desferrioxamine. These results indicate that Al has the potential to interact and disrupt A β peptide catabolism via the inhibition of proteolytic degradation.

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1. Introduction

There are many reports which show that Al causes inhibition of various protein functions and enzymes activities [1]. Particularly, Al seems to be a risk factor for various neurotoxic diseases caused by suppression of protein degradation [2]. Therefore, the influence of Al on protease activity is notable for the study of its neurotoxicity.

Earlier studies have suggested that Al inhibited calpain-mediated proteolysis of human neurofilament [3] and also inhibited trypsin and α -chymotrypsin proteolytic activity [4]. Korchazhkina et al. [5] recently reported that Al was able to inhibit plasmin degradation of the A β peptide (A β _{25–35}). Their results indicated that inhibitory effect of Al was caused by a direct interaction between Al and plasmin. Although many other A β degrading enzymes exert their action in extracellular media at neutral pH, some studies have demonstrated that A β peptide accumulation may occur within the intracellular matrix

[6–11]. This leads to the consideration that A β peptide is deposited and causes a core of neuritic plaques after cell death. Furthermore, Nakanishi described the pathological roles of neuronal and microglial cathepsins in brain aging and age-related diseases [12,13]. Indeed, recent reports indicated that A β peptide was taken up predominantly by microglia via class A scavenger receptors and the class B scavenger receptor type I [14,15]. Subsequently, the internalized A β peptides were accumulated and degraded in the lysosomes of microglia [16]. These observations strongly suggest that phagocytosed A β peptides are mainly degraded by cathepsin D in lysosomes. Cathepsin D is a typical aspartic protease in lysosome and functions primarily to degrade proteins by bulk proteolysis in the acidic milieu. Although the optimal pH of cathepsin D is approximately pH 3.5, it is likely that limited proteolysis is exerted by cathepsins in a less acidic intracellular compartment such as early and late endosomes [13].

Alternatively, previous studies using neuroblastoma cells [17] or rat cortical neurons [18] have described Al endocytosis with subsequent subcellular localization in lysosomes. Moreover, Xie et al. [19] reported that Al localized to the lysosomes in hippocampal pyramidal neurons in Al-loaded rabbits. We then considered that Al could exist as a soluble form when it is taken into the lysosome, which is a weakly acidic matrix. Therefore, it is possible that Al interacts with various proteins and internalized substrates within lysosomes.

In the present study, we focused on the effect of Al toward cathepsin D, a candidate enzyme in A β peptide degradation [20,21]. Our main objective was to evaluate whether Al might affect the proteolysis of proteins, including A β _{1–40} and A β _{1–42} peptide, by cathepsin D in an acidic matrix. Our overall purpose is to resolve a possible participation of Al in the accumulation of pathological proteins such as A β peptide and to propose a novel mechanism of Al-induced neurotoxicity.

2. Materials and methods

2.1. Direct effect of Al on cathepsin D activity

A synthetic peptide substrate used to assay of cathepsin D, (7-Methoxycoumarin-4-yl) acetyl-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys (Dnp)-D-Arg-NH₂, was purchased from Peptide Institute, Inc. (Osaka, Japan). This synthetic substrate was dissolved in DMSO and diluted to 0.2 mM in 50 mM acetate buffer (pH 4.0). Human liver cathepsin D (Calbiochem, CA, USA) was dissolved in 0.1 M 2-morpholinoethanesulfonic acid (MES) buffer (pH 4.5) at 18 U/ml. Five microliter of

*Corresponding author. Fax: +81 424 95 8529.

E-mail address: yo@my-pharm.ac.jp (Y. Ogasawara).

Abbreviations: AD, Alzheimer's disease; A β , amyloid β ; CD, circular dichroism; DFO, desferrioxamine; ICP-MS, inductively coupled plasma mass spectrometry; MES, 2-morpholinoethanesulfonic acid; TCA, trichloroacetic acid; TFA, trifluoroacetic acid

AlCl_3 solution (0.1–0.2 mM) was added to an equal volume of cathepsin D solution, and was incubated at 37 °C for 20 h. Following this, 5 μl of 0.2 mM synthetic peptide substrate and 35 μl of 50 mM acetate buffer (pH 4.0) were added to the cathepsin D and Al mixture, followed by incubation at 37 °C for 10 min. The reaction was stopped by rapid cooling on ice followed by the addition of an equal volume of ice-cold 5% trichloroacetic acid (TCA) solution. After centrifugation at $10000 \times g$ for 5 min, the fluorescence intensity of the supernatant was monitored at excitation and emission frequencies of 328 nm and 393 nm, respectively.

2.2. Indirect effect of Al on degradation of substrates by cathepsin D

2.2.1. Degradation of acid-denatured hemoglobin.

Acid-denatured hemoglobin, which is a typical substrate of cathepsin D, was prepared by the method of Schwabe [22]. Cathepsin D activity was determined by the method of Rosenfeld et al. [23] with minor modification. 20 μl of various concentrations of AlCl_3 solution was added to 0.5 ml of acetate buffer (pH 3.5) containing acid-denatured hemoglobin, and this was incubated at 37 °C for 30 min. Next, 20 μl of cathepsin D (25 U/ml) was added to the mixture, followed by incubation at 37 °C for 30 min. The reaction was stopped by the addition of an equal volume of ice-cold 5% TCA. After centrifugation at $10000 \times g$ for 5 min, the supernatant samples were measured by the Folin–Lowry method.

2.2.2. Degradation of $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ peptides.

When the $\text{A}\beta$ peptide was used as a substrate for cathepsin D, 0.5 mg/ml of synthetic $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ peptides (Bachem, Bubendorf, Switzerland) were incubated at 37 °C for 60 min with various concentration of AlCl_3 , FeCl_3 , and ZnCl_2 in 0.1 M MES buffer (pH 4.5). Next, 4.2 μl of 150 U/ml cathepsin D in 0.1 M MES buffer (pH 4.5) was added to 20 μl of the mixture. After further incubation at 37 °C for 20 h, the reaction was stopped by adding an equal volume of 1,1,1,3,3,3-hexafluoro-2-propanol, which acts as a strong denaturant of $\text{A}\beta$ peptides by breaking their steric structures and inducing the formation of α -helices [24] containing 0.2% trifluoroacetic acid (TFA). After centrifugation at 4 °C, $15000 \times g$ for 3 min at 4 °C, a portion of the supernatant was applied to reverse phase HPLC.

2.2.3. Determination of $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ peptides by HPLC.

The proteolytic degradation of $\text{A}\beta$ peptides was determined using reverse phase HPLC as described Hamazaki [20] and McDermott and Gibson [21] with minor modifications. We used an Inertsil 300 C_8 column (4.6×100 mm, 5 μm , GL Science, Tokyo, Japan) for $\text{A}\beta_{1-40}$ peptide, and a ZORBAX 300SB- C_{18} column (4.6×150 mm, 5 μm , Agilent, USA) for $\text{A}\beta_{1-42}$ peptide. To analyze $\text{A}\beta_{1-40}$ peptide degradation, the column temperature was maintained at 30 °C, with an injection volume of 50 μl . The $\text{A}\beta_{1-40}$ peptide was eluted at 26 min with a 40 min-linear gradient from 0% to 60% acetonitrile in 0.1% TFA using a flow rate of 1 ml/min; this was detected using UV absorbance at 215 nm. In contrast, determination of $\text{A}\beta_{1-42}$ peptide degradation was performed with the column temperature maintained at 40 °C; the injection volume remained at 50 μl . A mobile phase gradient was used for $\text{A}\beta_{1-42}$ peptide elution; the population of acetonitrile was increased from 20% to 60% over 25 min in 0.1% TFA using a flow rate of 1 ml/min, with UV detection at 220 nm. Under these conditions, $\text{A}\beta_{1-42}$ peptide was eluted in 15 min. All elutions were fractionated and applied to the protein sequencer to confirm the formation of amyloid fragments. Sequencing of peptides was performed using Protein Sequencer (PPSQ-21 A, Shimadzu, Japan).

2.3. Effect of DFO on the inhibition of $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ peptide degradation by Al

After preincubation of $\text{A}\beta$ peptides with Al, 1 mM desferrioxamine desferrioxamine (DFO) was added to the mixture. Following this, the degradation of the peptide was performed by adding cathepsin D and incubating for 20 h. Degradation of both $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ peptides in the presence and absence of DFO was observed by the HPLC method described above.

2.4. Circular dichroism spectroscopy

The circular dichroism (CD) spectrum was measured using a Jasco J-820 spectropolarimeter (Tokyo, Japan). The mixture containing 0.5 mM Al and 0.5 mg/ml $\text{A}\beta$ peptide in 0.1 M MES buffer (pH 4.5) was loaded into a cylindrical cell (0.5 mm path length) for measurements in the UV range of 190–260 nm (scan speed 20 nm/min). The nitrogen gas flow rate was set at 5 L/min. Repetitive scans were used

to improve the signal-to-noise ratio, and an average of four scans was performed.

2.5. Analysis of Al bound to various peptides

Ten microliter of 4 mg/ml $\text{A}\beta_{1-40}$ peptide, aprotinin (Sigma-Aldrich, MO, USA), or glucagon (Sigma-Aldrich) was added to 10 μl of 1.0 mM AlCl_3 in 0.1 M MES buffer (pH 4.5); the mixture was then incubated at 37 °C for 60 min. Next, the solution was applied to a polyvinylidene difluoride (PVDF) membrane cartridge (ProSorb, Applied Biosystems, USA) for a protein sequence assay apparatus. The membrane and inside of the tube were washed with 0.5 ml of 0.1 M MES buffer (pH 4.5) five times to remove excess free Al; Al bound to peptides on the PVDF membrane was recovered using 0.5 ml of 1 mM DFO aqua solution. Al content was determined by inductively coupled plasma mass spectrometry (ICP-MS) (Sciex Elan DRC II, Perkin-Elmer, USA).

2.6. Statistical analysis

All experimental data are shown as means \pm S.D. Data were analyzed using Student's *t*-test and analysis of variance (ANOVA) followed by Bonferroni correction. In all statistical analyses, the levels of significant differences were identified by $P < 0.05$.

3. Results

3.1. Direct effect of Al on cathepsin D activity

We evaluated the direct effect of Al on cathepsin D activity using synthetic low molecular weight substrate. When cathepsin D was preincubated with Al (0.125–0.5 mM) and then added to a synthetic low molecular weight substrate, cathepsin D activity was not significantly affected as compared to the control value generated without preincubation with Al (Fig. 1).

3.2. Influence of Al-pretreatment to substrates of cathepsin D

First, we examined the indirect influence of Al on cathepsin D activity using acid-denatured hemoglobin as the substrate. As shown in Fig. 2, after preincubation with 0.2, 0.5, and 1.0 mM Al for 30 min at 37 °C, the apparent degradation of acid-denatured hemoglobin was significantly decreased at $8.5 \pm 5.3\%$, $31.0 \pm 5.6\%$, and $72.8 \pm 6.8\%$ versus control ($n = 6$), respectively.

Subsequently, the effect of pretreatment with Al was examined using $\text{A}\beta$ peptides as substrates for degradation by cathepsin D. Chromatograms of $\text{A}\beta$ peptide clearly demonstrated that incubation with cathepsin D led to degradation

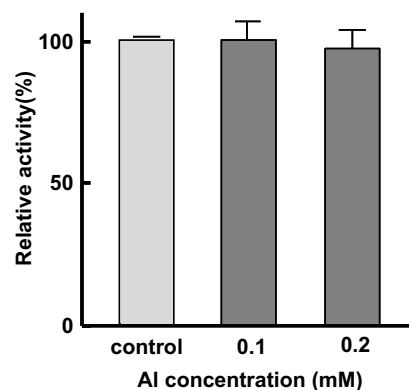


Fig. 1. The direct effects on cathepsin D activity by Al. The final Al concentrations were 0.1 and 0.2 mM in the reaction mixture. Analysis was performed to determine the fluorescence at an excitation wavelength of 328 nm and emission wavelength of 393 nm using a synthetic peptide substrate. Values are means \pm S.D. ($n = 4$).

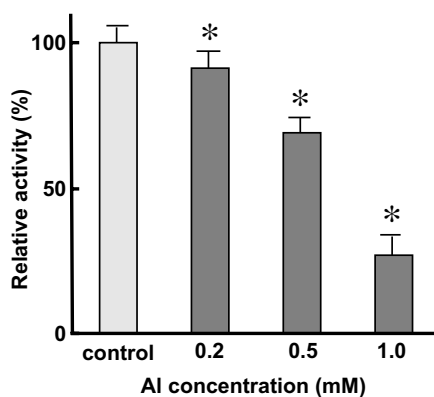


Fig. 2. Indirect effects of Al on degradation of acid-denatured hemoglobin by cathepsin D. The final Al concentrations were 0.2, 0.5, and 1.0 mM in the reaction mixture. Values are means \pm S.D. ($n = 6$). Significantly different from control; $P < 0.01$ (*).

of $A\beta_{1-40}$ (Fig. 3B) and $A\beta_{1-42}$ peptides (Fig. 4B). The digested fragments from each $A\beta$ peptide were recovered as shown in Figs. 3B and 4B. Although $A\beta_{1-19}$ and $A\beta_{20-34}$ were confirmed in the eluent from $A\beta_{1-40}$ peptide, only $A\beta_{1-19}$ was identified from $A\beta_{1-42}$ peptide under our HPLC conditions in order to quantify the amount of residual $A\beta_{1-40}$ or $A\beta_{1-42}$ peptide. However, when the each $A\beta$ peptide was pretreated with Al, Fe, or Zn (at a level about 0.25 mM), the degradation of $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides by cathepsin D was significantly inhibited by pretreatment of Al above 0.25 mM (Figs. 3C and 4C). In addition, the inhibitory effect of Al was more obvious than that of Fe and Zn (Tables 1 and 2). As shown in Table 1, when compared to the control, the degradation of the $A\beta_{1-40}$ peptide by cathepsin D was inhibited by $21.2 \pm 17.4\%$, $45.0 \pm 9.7\%$, and $46.9 \pm 15.9\%$ with pretreatment of Al at 0.125, 0.25, and 0.5 mM, respectively. Also, significant inhibition of the $A\beta_{1-42}$ peptide was observed when pretreated with Al at 0.25 mM and 0.5 mM (Table 2).

3.3. Influence of DFO on the inhibitory effect of Al for degradation of $A\beta$ peptides by cathepsin D

Degradation of the $A\beta_{1-40}$ peptide (Fig. 3D) and $A\beta_{1-42}$ peptide (Fig. 4D) by cathepsin D was recovered by addition of 1 mM DFO following pre-incubation of $A\beta$ with Al. The inhibition ratio induced by pretreatment with 0.5 mM Al was reduced from 46.9% to 10.4% for $A\beta_{1-40}$ peptide degradation using 1 mM DFO treatment, while the inhibitory effect caused by Al pretreatment on the degradation of $A\beta_{1-42}$ peptide was almost eliminated by treatment with 1 mM DFO.

3.4. Alteration of the secondary structure of $A\beta$ peptides induced by the pretreatment with Al

To investigate the mechanism of Al inhibitory of cathepsin D-mediated $A\beta$ peptide degradation, we performed a secondary structure analysis in solution using CD spectroscopy. Under the previously stated conditions, conformational changes were not observed in either the $A\beta_{1-40}$ or $A\beta_{1-42}$ peptides in the presence of 0.5 mM Al after at least 20 h of incubation at 37 °C, pH 4.5 (Fig. 5).

3.5. Binding of Al to $A\beta_{1-40}$ peptide

Furthermore, to investigate the mechanism by which Al inhibits the degradation of $A\beta$ peptide by cathepsin D, we at-

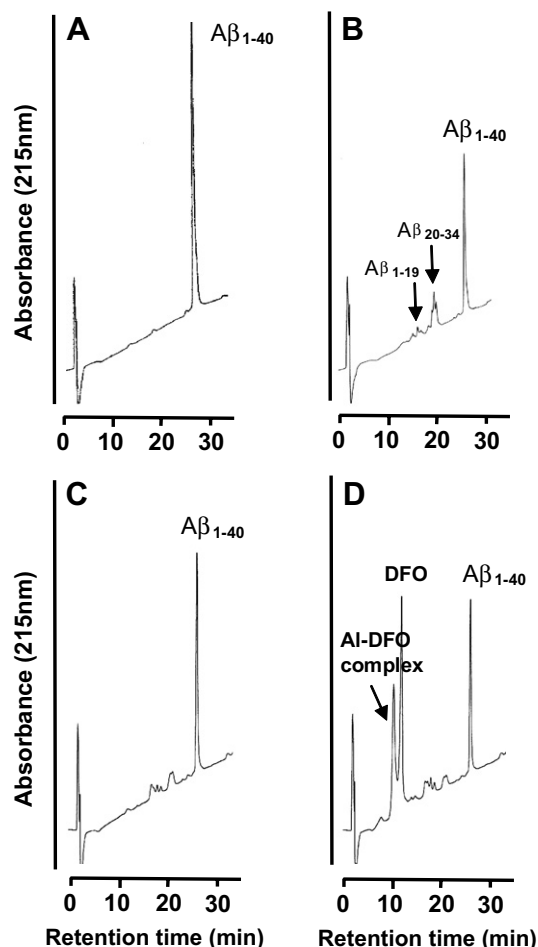


Fig. 3. Chromatograms of the degradation of $A\beta_{1-40}$ peptide by cathepsin D. (A) $A\beta_{1-40}$. (B) $A\beta_{1-40}$ digested by cathepsin D. (C) Pretreated $A\beta_{1-40}$ with 0.5 mM Al following digestion by cathepsin D. (D) Pretreated $A\beta_{1-40}$ with 0.5 mM Al followed by incubation with DFO for 30 min and subsequent digestion by cathepsin D. After incubation, the samples were analyzed on HPLC; all eluent (0–26 ml) were collected (1 ml/tube) for peptide identification. The new products of degradation were identified using protein sequencer.

tempted to demonstrate binding Al to the $A\beta$ peptide after incubation with $A\beta$ peptide under weakly acidic conditions (pH 4.5). In this experiment, we confirmed that free Al^{3+} was not precipitated by determining the concentration of Al in the filtered solution obtained from the control sample (with the vehicle containing 0.5 mM Al). Indeed, the control showed that free Al^{3+} was not retained on the membrane after washing with an acidic buffer (pH 4.5) (Fig. 6). Al bound to the $A\beta_{1-40}$ peptide, aprotinin, or glucagon on the PVDF membrane was released using DFO, and the recovered Al amounts were determined. As shown in Fig. 6, the Al content bound to the $A\beta_{1-40}$ peptide was more significant than that of aprotinin and glucagon.

4. Discussion

$A\beta$ peptides are core constituents of the neuritic (senile) plaques, one of the typical neuropathological changes of Alzheimer's disease (AD) [25,26]. It has been widely viewed that AD might arise from an imbalance in the rate of synthesis

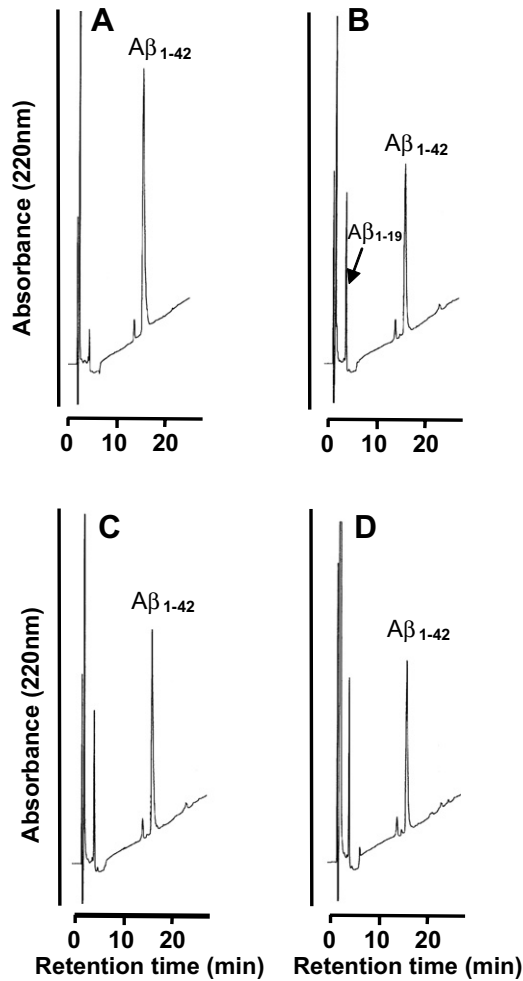


Fig. 4. Chromatograms of the degradation of A β_{1-42} peptide by cathepsin D. (A) A β_{1-42} . (B) A β_{1-42} digested by cathepsin D. (C) Pretreated A β_{1-42} with 0.5 mM Al followed by digestion with cathepsin D. (D) Pretreated A β_{1-42} with 0.5 mM Al followed by incubation with DFO for 30 min and subsequent digestion by cathepsin D. After incubation the samples were analyzed on HPLC; all eluent (0–15 ml) were collected (1 ml/tube) for peptide identification. The new products of degradation were identified using protein sequencer.

Table 1
Inhibitory effect of Al, Fe, and Zn on A β_{1-40} peptide degradation by cathepsin D

Concentration (mM)	Inhibition (%)		
	Al	Fe	Zn
0.125	21.2 \pm 17.4	–	–
0.25	45.0 \pm 9.7 **	1.1 \pm 1.9	0.2 \pm 0.3
0.50	46.9 \pm 15.7 **	38.9 \pm 7.3 *	5.8 \pm 5.4

Each data represents mean values \pm S.D. ($n = 3-5$). The inhibition (%) of degradation by Al, Fe, and Zn are calculated by dividing the peak area of A β_{1-40} peptide in the presence of each metal by the corresponding peak areas obtained in the absence of each metal. Significant differences from control value: (–) indicates not done.

* $P < 0.05$.

** $P < 0.001$.

versus the rate of clearance of A β peptide. In physiologically normal metabolism, A β peptide levels appear to be strictly regulated, resulting in a low level of A β peptide and no deposition

Table 2
Inhibitory effect of Al, Fe, and Zn on A β_{1-42} peptide degradation by cathepsin D

Concentration (mM)	Inhibition (%)		
	Al	Fe	Zn
0.125	≤ 0	–	–
0.25	36.7 \pm 13.7 *	≤ 0	≤ 0
0.50	36.7 \pm 8.4 **	≤ 0	≤ 0

Each data represents mean values \pm S.D. ($n = 3-5$). The inhibition (%) of degradation by Al, Fe, and Zn are calculated by dividing the peak area of A β_{1-42} peptide in the presence of each metal by the corresponding peak areas obtained in the absence of each metal. Significant differences from control value: (–) indicates not done.

* $P < 0.05$.

** $P < 0.001$.

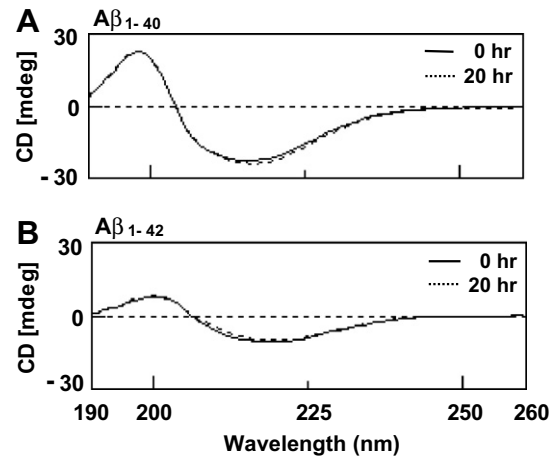


Fig. 5. The effect of Al on CD spectrum of A β_{1-40} and A β_{1-42} peptide under acidic condition. A β peptides were incubated with 0.5 mM Al at pH 4.5 (0.1 M MES buffer). Each reaction mixture was measured on time (0 h) and after 20 h incubation at 37 °C. Typical CD spectra for (A) A β_{1-40} and (B) A β_{1-42} are shown.

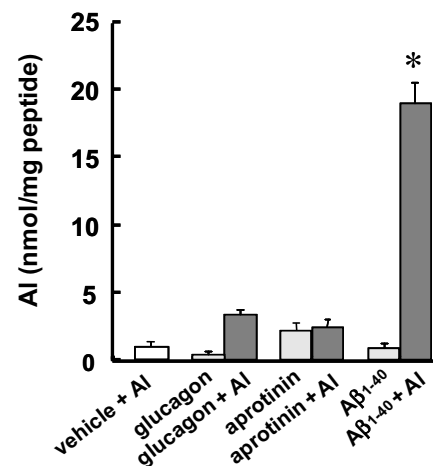


Fig. 6. Binding of Al to various peptides. Each peptide was incubated with Al at pH 4.5, thereafter the reaction mixture was then applied to a PVDF membrane for protein sequence assay. Al bound to A β_{1-40} , aprotinin, and glucagon on the PVDF membrane was recovered by 1 mM DFO solution, and the Al content was determined by ICP-MS. The procedure for determining Al concentration is described in Section 2. Each data represents mean values \pm S.D. ($n = 4$). Significantly different from ANOVA analyses; $P < 0.01$ (*).

in the brain. Therefore, it is considered that a failure in a proteolytic function causes accumulation of A β peptide. Several proteases have been shown to be capable of cleaving A β peptide. There are number of candidate proteases, including trypsin and α -chymotrypsin [27], insulin degrading enzyme [28–30], endothelin-converting enzyme [31], carboxypeptidase B [32], angiotensin-converting enzyme [33], plasmin [34,35], neprilysin [36] and cathepsin D [20,21]. However, the enzyme definitively responsible for the physiological degradation of A β peptide is thus far unclear.

Because soluble Al³⁺ is markedly increased in acidic matrices, we examined the influence of Al on the activity of lysosomal cathepsin D-mediated proteolysis of A β peptides. When a synthetic decapeptide substrate was used, direct interaction was not observed between Al and human cathepsin D. In addition, Falkous et al. [37] did not find significant direct effects of Al to lysosomal protease when synthetic small peptides were used as substrate. However, degradation of acid-denatured hemoglobin and A β peptides by cathepsin D was significantly inhibited when Al was pretreated with the cathepsin D substrates. This apparent inhibition likely indicates that acid-denatured hemoglobin interacts with Al³⁺ to form complexes which are not easily degraded by cathepsin D. Therefore, we further examined the potential of Al to induce substrate intensively toward decomposition by cathepsin D using A β peptides.

We observed the inhibitory effects of Fe and Zn as well as Al on the A β peptide degradation by cathepsin D using HPLC [20,21]. Sample preparation containing 1,1,1,3,3,3-hexafluoro-2-propanol and TFA gave good recovery of A β peptides without aggregation. A β _{1–40} and A β _{1–42} peptides could be detected quantitatively HPLC under conditions used in this study. Fe and Zn showed only a slight inhibitory effect on A β peptide degradation when compared to Al under same conditions. Although Mantyh et al. [38] and Kawahara et al. [39–41] demonstrated that Al, Fe, and Zn enhanced the aggregation of the A β _{1–40} peptide, we did not observe aggregation of the A β peptides following their incubation with 0.5 mM Al for 20 h at 37 °C, pH 4.5. Thus, based on the observed interaction between Al and A β peptides, the inhibitory effect of Al appears to be specific for acidic conditions.

In order to evaluate the apparent inhibitory effect of Al for A β degradation by cathepsin D, we first estimated whether Al treatment caused a conformational change in the A β peptides, resulting in resistance towards cathepsin D. Some researchers have reported that Al accelerated the conformational changes of A β peptide from random to β -sheet form [42–44]. We observed the conformation of A β _{1–40} and A β _{1–42} peptides in the presence of Al under acidic conditions and no changes in secondary structure of A β were observed, at least within the 20 h of incubation at pH 4.5. However, our reaction conditions (e.g. pH and incubation time) differ from that done in previous studies.

Next, we investigated another inhibitory mechanism by which Al could inhibit A β peptide degradation by cathepsin D. From the results shown in Tables 1 and 2, A β peptide seems to be tolerant to cathepsin D when bound to Al under soluble conditions. Addition of DFO prior to cathepsin D degradation caused a disappearance of the inhibitory effect by Al on A β peptides degradation; this suggests that Al exerted its effects via reversible interaction with the A β peptide. This result also indicates that a chelating agent like Clioquinol [45,46] may be

effective for the treatment of Alzheimer's disease, if Al participates in the pathophysiology of the disease. Since these results strongly indicate that Al associated with A β as soluble complex at pH 4.5, we attempted to determine if a significant amount of Al bound to the peptides using a PVDF membrane technique. In general, the propensity for Al³⁺ to be bound by biological ligands such as carboxylate at acidic pH will be lower than at neutral pH. However, our results clearly demonstrated that Al bound easily to A β _{1–40} peptides at a physiologically-relevant pH 4.0–5.0 (i.e. that of the lysosomal fraction in the brain). Recently, Vyas et al. [47] indicated using ²⁷Al nuclear magnetic resonance spectroscopy and amino acid sequencing of A β peptides in the presence of Al that Al³⁺ ions could interact with A β _{1–40} and A β _{6–25} peptides. Their data also showed the possibility that Asp⁷, His¹⁴, and Asp²³ have undergone steric interference associated with aqueous Al³⁺ to form a relatively stable complex. Moreover, they revealed that the A β peptide is stably bound by Al at pH 4.0–6.0 via its sites at Asp⁷, His¹⁴, and Asp²³, which undergo steric interference Al³⁺ ion-complexation. This observation supports our result that Al has binding potential to the A β peptides in acid matrix. On the other hand, cathepsin D cleaves the A β peptide at Phe¹⁹-Phe²⁰ and Leu³⁴-Met³⁵ [20,21]. Therefore, it is possible that the steric interaction between Al and A β peptides may affect the digestion of A β peptides by disturbing the cleavage sites for cathepsin D, even before aggregation of the A β peptide occurs. As the resultant, it can be considered that Al induces the deposition of A β peptides via its inhibitory potential against A β degradation by cathepsin D.

In a previous report, we showed that the relationship between Al accumulation and the acceleration of lipid peroxidation in the rat brain [48]. Interestingly, recent studies clearly suggest that neurotoxicity exerted by senile plaques arises from the induction of oxidative stress [49–51]. Furthermore, Pratico et al. [52] reported that Al increased in vivo lipid peroxidation, as well as Al-accelerated A β peptide formation and plaque deposition in amyloid precursor protein transgenic mice. Therefore, it seems important to establish the relationship between the acceleration of lipid peroxidation and plaque deposition induced by Al.

In conclusion, Al inhibits the degradation of some substrates of cathepsin D. Therefore, it is proposed that Al triggers the intracellular accumulation of proteins and peptides, including A β peptide, if cathepsin D participates its degradation in vivo. Although the mechanisms of AD pathogenesis have not been fully elucidated, increasing evidence indicates that the accumulation of A β peptides might cause the formation of the neuritic plaques, and the neurotoxicity that results are due to the generation of radical oxygen species [49–51]. Thus, the inhibitory potential of Al towards cathepsin D presented here can be considered a toxic feature of Al, which may be a risk factor of AD-like pathogenesis through the deposit of neuritic plaque.

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