— **Minireview** —

Aluminum-Induced Conformational Changes of β-Amyloid Protein and the Pathogenesis of Alzheimer's Disease

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Aggregation and subsequent conformational change of Alzheimer's β-amyloid protein (AβP) enhance its neurotoxicity. Therefore, factors that inhibit or promote conformational changes of AβP play crucial roles in the pathogenesis of Alzheimer's disease (AD). Moreover, recent studies have suggested that a common mechanism is based on the diverse diseases termed "conformational diseases" including neurodegenerative diseases such as AD, prion diseases, Parkinson's disease, and Huntington's disease. These diseases share similarity in the formation of β-sheet containing amyloid fibrils by disease-related proteins and the introduction of apoptotic degeneration. Aluminum, an environmental risk factor for AD, is a widely used cross-linker that causes conformational changes of AβP and other proteins. This report reviews and discusses characteristics of aluminum-induced conformational changes of AβP and their implication in pathogenesis of AD. Taking together our results and those of numerous other studies, we hypothesize that aluminum-induced conformational changes enhance the neurotoxicity of AβP and lead to development of AD.

Key words —— conformational disease, prion disease, aggregation, neurotoxicity, calcium homeostasis

INTRODUCTION

Alzheimer's disease (AD) is a senile type dementia characterized by abnormal deposits of senile plaques and neurofibrillary tangles (NFTs) in patient's brain. The major component of senile plaque is β-amyloid protein (AβP). AβP is a small peptide with 39–43 amino acid residues derived from the proteolytic cleavage of a large precursor protein (amyloid precursor protein; APP). Yankner *et al*. reported that the first 40 amino acid residues of AβP (AβP[1–40]) caused the death of cultured rat hippocampal neurons.[1] Recent genetic studies revealed that the 21st chromosome of familial Alzheimer's patients possesses a single point mutation in the codon for APP.[2] These evidences support the idea that accumulation of AβP and consequent neurodegeneration caused by AβP may be based on the molecular mechanism of AD.[3] However, AβP has an intrinsic tendency to polymerize and form insoluble aggregates with β-pleated sheet structures in an aqueous solution. There is increasing evidence that aggregation and subsequent conformational changes of AβP enhance its neurotoxicity. Simmons *et al*. demonstrated that the ratio of β-sheet structures of AβP[1–40] was correlated with its neurotoxicity.[4] A longer peptide variant (AβP[1–42]) has the characteristic of immediate polymerization compared to AβP[1–40]: it enhances aggregation of AβP[1–40].[5]

Considering that AβP exists in cerebrospinal fluid (CSF) even during childhood and that its concentration is not elevated in the CSF of AD patients compared with that in controls,[6] it is probable that factors that promote or inhibit aggregation of AβP play crucial roles in its neurotoxicity (Fig. 1). As such inhibitory factors, transthyretin, a protein colocalized with AβP in CSF, was reported to bind to AβP and thereby inhibit its aggregation.[7] Non-aggregated AβP

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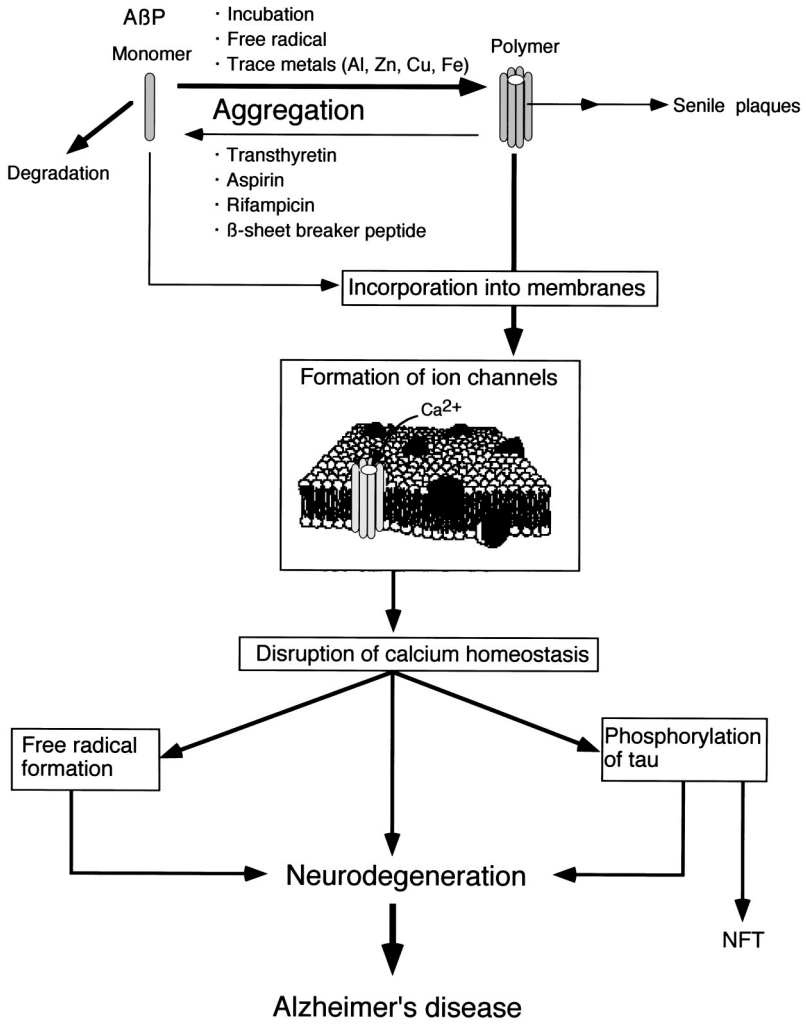

Fig. 1. Hypothesis Regarding the Metal-Induced Aggregation of AβP in the Pathogenesis of Alzheimer's Disease

Secreted AβP is rapidly degraded by proteases in general. However, in the presence of factors that promote aggregation, AβP forms insoluble and protease-resistant polymers and accumulates in the brain. AβP can incorporate into membranes directly, where it forms "amyloid channels" and disrupts calcium homeostasis. The channels comprise peptide oligomers; therefore, polymerization of AβP enhances the provability of forming channels. The abnormal influx of calcium through the amyloid channels can trigger various neurodegenerative pathways such as the phosphorylation of tau and the generation of free radicals, leading to neuronal death and AD pathogenesis. The abnormal calcium influx through amyloid channels causes neuronal death and may lead ultimately to AD.

is inferred to be degraded by the usual proteolytic pathways. Several non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen were reported to inhibit the aggregation of AβP.[8] Recent epidemiological studies have suggested that these NSAIDs reduce the relative risk of AD.[9] Rifampicin (an antibiotic), or β-sheet breaker peptide (a peptide analogue of AβP) were also reported to inhibit polymerization of AβP and are considered to be candidates of drugs for AD treatment.[10,11] On the other hand, there are factors that promote the polymerization of AβP and thereby enhance the risk of AD. As such factors, free radicals,[12] incubation at 37°C,[13] and trace metals have been reported. Among them, aluminum (Al) has attracted attention for its possible implication in AD based on numerous epidemiological and biochemical studies.[14]

Effects of Aluminum on Conformational Changes of AβP

The aluminum ion (Al^{3+}) possesses several peculiar chemical characteristics.[15] Al^{3+} favors oxygen-donor ligands, especially if they are negatively charged. Inorganic or organic phosphates, carboxylate, deprotonated hydroxy groups are strong Al^{3+} binders. Furthermore, the ligand-exchange rate for Al^{3+} is very low compared to other essential metals. For example, Al^{3+} is reported to be 10^7 more effec-

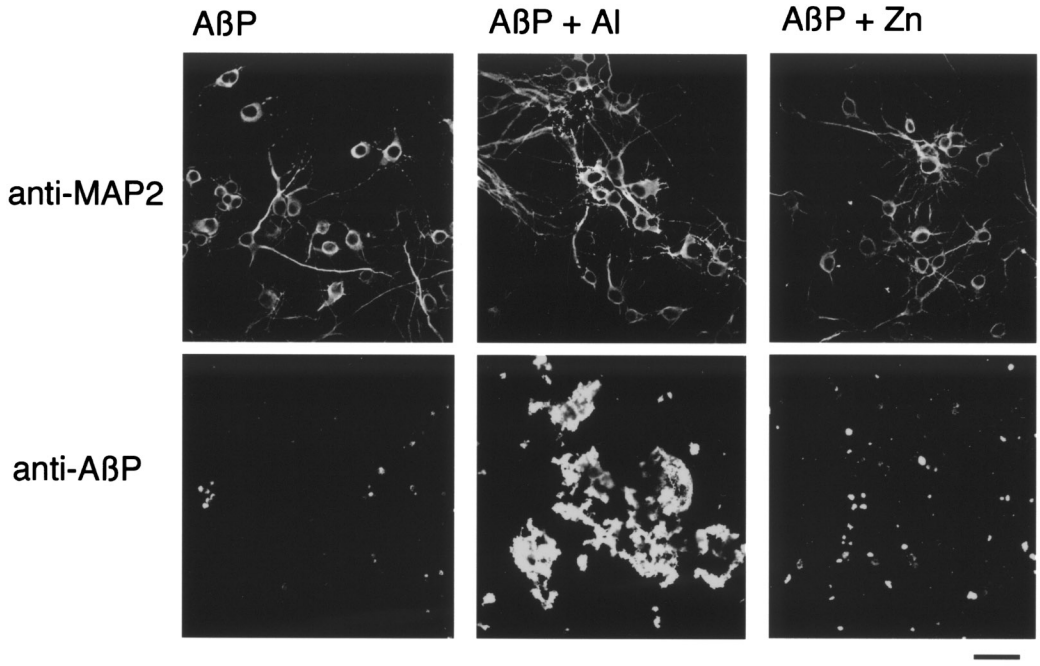

Fig. 2. Deposition of Al-Aggregated AβP on Cultured Neurons

Solutions of AβP[1–40] pre-incubated at 37°C for 24 hr, AβP[1–40] pretreated with $AlCl_3$ 1 mM, and AβP[1–40] pretreated with $ZnCl_2$ 1 mM were applied to cultured rat cortical neurons. After 4 days, cultured neurons were washed out, double-immunostained with an antibody to AβP and an antibody to MAP2, and observed under a confocal laser scanning microscope. Bar represents 50 μm.

tive than Mg^{2+} in the binding and polymerization of tubulin. Thus, biological processes involving rapid Ca^{2+} exchange could be inhibited by substitution of the 10^8-fold slower Al^{3+}. Also, Al^{3+} shares similarities with iron ion (Fe^{3+}) and binds to Fe^{3+}-binding proteins such as transferrin. These chemical characteristics of Al^{3+} make it useless in metal-engaged biological reactions, and therefore, Al is not essential in biological functions. Al is a widely used cross-linker as a tanning agent for leathers.

There are reports of Al^{3+}-induced conformational change of calmodulin.[16] Strong binding of Al^{3+} to phosphorylated amino acids promotes self-aggregation of highly phosphorylated cytoskeletal proteins such as neurofilament or microtubule-associated proteins (MAPs).[17,18] Al^{3+} also binds to transferrin and changes its conformation. Exley *et al*. demonstrated that Al caused conformational changes of AβP[1–40] using circular dichroism spectroscopy.[19] Mantyh *et al*. reported that Al, Fe and zinc (Zn) promoted aggregation of ^{125}I-labeled AβP[1–40].[20] We have developed a system for the investigation of AβP polymerization involving immunoblotting and precipitation. Using that method, we found that Al enhances polymerization of AβP[1–40] and forms sodium dodecyl sulfate (SDS)-stable oligomers *in vitro*.[21,22] Aggregated AβP[1–40] was re-dissolved by adding deferoxamine (DFO), a chelator of Al. Polymerization induced by Al was more marked than that by other metals including Zn, Fe, Cu, and cadmium (Cd). Furthermore, Al-aggregated AβPs tightly bound to the surface of cultured neurons and formed fibrillar deposits, whereas Zn-aggregated AβPs were rarely observed on the surface of cultured neurons after 4 days (Fig. 2).[23] These results suggest that Al-aggregated AβPs have strong affinity to membrane surfaces and are scarcely degraded by proteases. Although Al^{3+} firmly binds to adenosine triphosphate (ATP), ATP enhanced Al-induced β-sheet formation of AβP.[24] Meanwhile, acetyl salicylic acid (aspirin) reduced Al-induced aggregation of AβP.[8]

Bush *et al*. argued that Zn^{2+} in the range similar to that in CSF was sufficient to cause aggregation of AβP.[25] The binding of Cu^{2+} to AβP induced aggregation and caused rise to reactive oxygen species.[26] Furthermore, APP possesses copper/zinc binding sites in its amino-terminal domain and in the AβP domain.[27] However, a large amount of Zn exists in the brain and the high concentration (up to 300 μM) of Zn^{2+} is secreted to synaptic clefts after neuronal excitation.[28] Thus, the implication of Zn-induced AβP polymerization in the etiology of AD remains elusive.

Abnormal deposits of AβP (senile plaques) are

observed in aged humans, monkeys, and dogs, but rarely found in rats and mice. Although the sequences of rodent APP are approximately 96% similar to human APP, three amino acid substitutions are found in the AβP region of rodents: $Arg_5 \rightarrow Gly$, $Tyr_{10} \rightarrow Phe$, and $His_{13} \rightarrow Arg$.[29] All of these residues have the ability to bind to metals. The Tyr and Arg residues of transferrin are essential for its binding to Fe^{3+}. Aggregation of AβP induced by Zn or Cu was abolished by the methylation of its His residues.[27] Therefore, it is probable that these metal-binding amino acid residues are crucial for metal-induced polymerization of AβP; their substitutions might explain the lack of amyloid deposits in rodent brains. We synthesized mutated AβP substituted these metal-binding amino acid residues to investigate the mechanism of Al^{3+} binding to AβP. We found that Tyr_{10} is essential for Al-induced polymerization of AβP.[30]

Effects of Aluminum on Other Disease-Related Proteins

Diverse human disorders are thought to arise from the misfolding and aggregation of an underlying protein. Those diseases include neurodegenerative disorders such as AD, Parkinson's disease, prion encephalopathies, and triplet repeat disease (including Huntington's disease), as well as cystic fibrosis and systemic amyloidosis. Carrell and Lomas proposed the concept of "conformational disease," which may explain the same mechanism of development of these diseases.[31] The underlying disease-related proteins exhibit similarities in terms of formation of amyloid fibrils with β-pleated sheet structures and the introduction of apoptotic degeneration. Aggregation and fibrillation of α-synuclein has been implicated in the formation of abnormal inclusions (Lewy bodies) and has been considered as a key step in the etiology of Parkinson's disease and several other neurodegenerative disorders (dementia with Lewy bodies; DLB). Uversky *et al*. found that Al and other metals markedly promoted the aggregation of α-synuclein.[32] Furthermore, Al promotes aggregation of APP[33] and tau protein,[34] which is a major component of Alzheimer's NFTs.

Mechanism of AβP Neurotoxicity

The precise mechanism underlying AβP-induced neuronal death remains elusive. However, we have found that AβP[1–40] directly incorporates into neuronal membranes and forms cation-selective (including Ca^{2+}) ion channels.[35] Characteristics of these amyloid channels ("pores") formed on neuronal membranes were quite similar to those first reported on artificial planar membranes.[36,37] Conformational analysis of AβP in membranes suggests that the 5- to 8-mers of AβP[1–40] oligomerize and form a channel structure across the membrane.[38] AβP[1–42] or other amyloidogenic peptides such as prion protein fragment in prion diseases, human islet amyloid peptide (amylin) in type 2 *diabetes mellitus*, polyglutamin in triplet repeat disease, and α-synuclein in Parkinson's disease were reported to form channels on artificial lipid bilayers.[39] Table 1 summarizes the characteristics of amyloidogenic peptides. Although these peptides have no similarities in their primary sequences, all peptides form amyloid fibril structures with β-pleated sheets, possess cytotoxicity, and have ability to form ion channels. Meanwhile, although the sequence of rodent amylin is approximately 95% similar to human amylin, rat amylin has no ability to form amyloid fibrils, to cause cytotoxicity, or to form ion channels.

Abnormal influx of Ca^{2+} into neurons through these "amyloid channels" is inferred to trigger various neurodegenerative processes and apoptotic pathways. In fact, we have also demonstrated that AβP causes an acute increase of intracellular calcium level of neuronal cells and consequent cell death as expected. Furthermore, other amyloidogenic peptides including prion protein fragment and human islet amyloid peptide caused disruption of calcium homeostasis.[40]

In light of these facts, we formed the following hypothesis about the polymerization of AβP and Alzheimer's pathogenesis: Once channels were formed on neuronal membranes, the abnormal influx of $[Ca^{2+}]$ triggers various neurodegenerative pathways, and finally leads to AD. This hypothesis coincides with the idea that amyloid fibers form cylindrical nanotubes constructed with β-sheet oligomers as shown by X-ray crystallography and electron microscope analysis.[41]

CONCLUSION

Although the link between Al and AD has been discussed for several decades, it is still controversial. However, there is increasing evidence that suggests the implication of metals in AD pathogenesis.

Table 1. Characteristics of Conformational Disease-Related Protein

Disease	Amyloidogenic protein or its fragment peptide	β-sheet formation	Cyto toxicity	Channel formation
Alzhelmer's disease	AβP[1–40] DAEFRHDSGYEVHHQKLVFFAE DVGSNKGAIIGLMVGGVV	+	+	+
Prion disease	PrP106–126 (prion protein fragment) KTNMKHMAGAAAAGAVVGGLG	+	+	+
Parkinson's disease	NAC (α-synuclein fragment) EQVTNVGGAVVTGVTAVAQKTVEGAG SIAAATGFV	+	+	+
Triplet repeat disease	Polyglutamine QQQQQQ——	+	+	+
Diabetes mellitus	Human amylin KCNTATCATQRLANFLVHSSNN FGAILSSTNVGSNTY	+	+	+
	Rat amylin KCNTATCATQRLANFLVRSSNN LGPVLPPTNVGSNTY	−	−	−

A considerable number of epidemiological studies has indicated an association between AD and Al in drinking water.[42] Recent studies have suggested that clioquinol (quinoform), a metal chelator, was reported to inhibit accumulation of AβP and has been examined as a candidate drug for AD.[43] Heme deficiency induced by aging, iron-deficiency, vitamin B6-deficiency, metals such as lead (Pb) or Al causes mitochondrial and neuronal dysfunction.[44] The polymorphism of the gene encoding transferrin is a risk factor for AD.[45] Iron supplement therapy is effective in attenuating the mental condition of AD patients.[46] Al in the diet markedly increased the amount of secreted AβP in the brains of transgenic mice transfected with the human APP gene; AβP accumulation was also marked.[47] Furthermore, we have reported that Al influences the functions of brain-derived neurotrophic factor which decreses in AD brain.[48] Therefore, it is difficult to deny the implication of Al in AD pathogenesis. Our hypothesis suggesting that Al induces conformational changes of AβP and enhances the neurotoxicity could explain several unsolved aspects of the relation between Al and AD.

REFERENCES

1) Yankner, B. A., Duffy, L. K. and Kirschner, D. A. (1990) Neurotropic and neurotoxic effects of amyloid β protein: reversal by tachykinin neuropeptides. *Nature* (London), **250**, 279–282.
2) Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N. and James, L., *et al*. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* (London), **349**, 704–706.
3) Small, D. H., Mok, S. S. and Bornstein, J. C. (2001) Alzheimer's disease and Aβ toxicity: from top to bottom. *Nat. Rev. Neurosci*., **2**, 595–598.
4) Simmons, L. K., May, P. C., Tomaselli, K. J., Rydel, R. E., Fuson, K. S., Brigham, E. F., Wright, S., Lieberburg, I., Becker, G. W. and Brems, D. N., *et al*. (1994) Secondary structure of amyloid beta peptide correlates with neurotoxic activity *in vitro*. *Mol. Pharmacol*., **45**, 373–379.
5) Jarrett, J. T. and Lansbury, P. T., Jr. (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell*, **73**, 1055–1058.
6) Fukuyama, R., Mizuno, T., Mori, S., Nakajima, K., Fushiki, S. and Yanagisawa, K. (2000) Age-dependent change in the levels of Aβ40 and Aβ42 in cerebrospinal fluid from control subjects, and a decrease in the ratio of Aβ42 to Aβ40 level in

cerebrospinal fluid from Alzheimer's disease patients. *Eur. Neurol*., **43**, 155–160.
7) Schwarzman, A. L., Gregori, L., Vitek, M. P., Lyubski, S., Strittmatter, W. J., Enghilde, J. J., Bhasin, R., Silverman, J., Weisgraber, K. H. and Coyle, P. K., *et al*. (1994) Transthyretin sequesters amyloid beta protein and prevents amyloid formation. *Proc. Natl. Acad. Sci. U.S.A*., **91**, 8368–8372.
8) Thomas, T., Nadackal, T. G. and Thomas, K. (2001) Aspirin and non-steroidal anti-inflammatory drugs inhibit amyloid-beta aggregation. *Neuroreport*, **12**, 3263–3267.
9) Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M. and Stricker, B. H. (2001) Nonsteroidal anti-inflammatory drugs and the risk of Alzheimer's disease. *N. Engl. J. Med*., **345**, 1515–1521.
10) Tomiyama, T., Asano, S., Suwa, Y., Morita, T., Kataoka, K., Mori, H. and Endo, N. (1994) Rifampicin prevents the aggregation and neurotoxicity of amyloid beta protein in vitro. *Biochem. Biophys. Res. Commun*., **204**, 76–83.
11) Permanne, B., Adessi, C., Saborio, G. P., Fraga, S., Frossard, M. J., Van Dorpe, J., Dewachter, I., Banks, W. A., Van Leuven, F. and Soto, C. (2002) Reduction of amyloid load and cerebral damage in a transgenic mouse model of Alzheimer's disease by treatment with a beta-sheet breaker peptide. *FASEB. J*., **16**, 860–862.
12) Hensley, K., Carney, J. M., Mattson, M. P., Aksenova, M., Harris, M., Wu, J. F., Floyd, R. A. and Butterfield, D. A. (1994) A model for β-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*., **91**, 3270–3274.
13) Cotman, C. W., Pike, C. J. and Copani, A. (1992) β-Amyloid neurotoxicity: a discussion of *in vitro* findings. *Neurobiol. Aging*, **13**, 587–590.
14) Kawahara, M. (2001) Neurotoxicity of aluminum and its implication in neurodegenerative disease. *Biomed. Res. Trace Elements*, **12**, 207–216.
15) Martin, R. B. (1997) Chemistry of aluminum in the central nervous system. In *Mineral and Metal Neurotoxicology* (Yasui, M., *et al*., Eds.) CRC Press, New York, pp. 75–81.
16) Siegel, N. and Haug, A. (1983) Aluminum interaction with calmodulin. Evidence for altered structure and function from optical and enzymatic studies. *Biochim. Biophys. Acta*, **744**, 36–45.
17) Diaz-Nido, J. and Avila, J. (1990) Aluminum induces the *in vitro* aggregation of bovine brain cytoskeletal protein. *Neurosci. Lett*., **110**, 221–226.
18) Hollosi, M., Urge, L., Perczel, A., Kajtar, J., Teplan, I., Otvos, L., Jr. and Fasman, G. D. (1994) Metal ion-induced conformational changes of phosphorylated fragments of human neurofilament (NF-M) protein. *J. Mol. Biol*., **223**, 673–682.
19) Exley, C., Price, N. C., Kelly, S. M. and Birchall, J. D. (1993) An interaction of β-amyloid with aluminium *in vitro*. *FEBS Lett*., **324**, 293–295.
20) Mantyh, P. W., Ghilardi, J. R., Rogers, S., DeMaster, E., Allen, C. J., Stimson, E. R. and Maggio, J. E. (1993) Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of β-amyloid peptide. *J. Neurochem*., **61**, 1171–1174.
21) Kawahara, M., Muramoto, K., Kobayashi, K., Mori, H. and Kuroda, Y. (1994) Aluminum promotes the aggregation of Alzheimer's β-amyloid protein *in vitro*. *Biochem. Biophys. Res. Commun*., **198**, 531–535.
22) Kuroda, Y. and Kawahara, M. (1994) Aggregation of amyloid β-protein and its neurotoxicity: enhancement by aluminum and other metals. *Tohoku J. Exp. Med*., **174**, 263–268.
23) Kawahara, M., Kato, M. and Kuroda, Y. (2001) Effects of aluminum on the neurotoxicity of primary cultured neurons and on the aggregation of β-amyloid protein. *Brain Res. Bull*., **55**, 211–217.
24) Exley, C. (1997) ATP-promoted amyloidosis of an amyloid beta peptide. *Neuroreport*, **8**, 3411–3414.
25) Bush, A. I., Pettingell, W. H., Multhaup, G., d Paradis, M., Vonsattel, J. P., Gusella, J. F., Beyreuther, K., Masters, C. L. and Tanzi, R. E. (1994) Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science*, **265**, 1464–1467.
26) Atwood, C. S., Moir, R. D., Huang, X., Scarpa, R. C., Bacarra, N. M., Romano, D. M., Hartshorn, M. A., Tanzi, R. E. and Bush, A. I. (1998) Dramatic aggregation of Alzheimer Aβ by Cu(II) is induced by conditions representing physiological acidosis. *J. Biol. Chem*., **273**, 12817–12826.
27) Curtain, C. C., Ali, F., Volitakis, I., Cherny, R. A., Norton, R. S., Beyreuther, K., Barrow, C. J., Masters, C. L., Bush, A. I. and Barnham, K. J. (2001) Alzheimer's disease Amyloid-β binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. *J. Biol. Chem*., **276**, 20466–20473.
28) Frederickson, C. J., Suh, S. W., Silva, D., Frederickson, C. J. and Thompson, R. B. (2000) Importance of zinc in the central nervous system: the zinc-containing neuron. *J. Nutr*., **130**, 1471S–1483S.
29) Fraser, P. E., Nguyen, J. T., Inouye, H., Surewicz, W. K., Selkoe, D. J., Podlisny, M. B. and Kirschner,

D. A. (1992) Fibril formation by primate, rodent, and Dutch-hemorrhagic analogues of Alzheimer amyloid beta-protein. *Biochemistry*, **31**, 10716–10723.
30) Kawahara, M., Kojima, S., Kato, M., Mori, H., Miura, K. and Kuroda, Y. (1995) Effects of site-specific mutagenesis on the aggregation of amyloid β-protein (AβP): interactions with aluminum. *Abst. Soc. Neurosci.*, **21**, 155.
31) Carrell, R. W. and Lomas, D. A. (1997) Conformational disease. *Lancet*, **350**, 134–138.
32) Uversky, V. N., Li, J. and Fink, A. L. (2001) Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular link between Parkinson's disease and heavy metal exposure. *J. Biol. Chem.*, **276**, 44284–44296.
33) Chong, Y. H. and Suh, Y. H. (1995) Aggregation of amyloid precursor proteins by aluminum *in vitro*. *Brain Res.*, **670**, 137–41.
34) Scott, C. W., Fieles, A., Sygowski, L. A. and Caputo, C. B. (1993) Aggregation of tau protein by aluminum. *Brain Res.*, **628**, 77–84.
35) Kawahara, M., Arispe, N., Kuroda, Y. and Rojas, E. (1997) Alzheimer's disease amyloid β-protein forms Zn^{2+}-sensitive, cation-selective channels across excised membrane patches from hypothalamic neurons. *Biophys. J.*, **73**, 67–75.
36) Arispe, N., Rojas, E. and Pollard, H. B. (1993) Alzheimer disease amyloid beta protein forms calcium channels in bi-layer membranes: Blockade by tromethamine and aluminum. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 567–571.
37) Arispe, N., Pollard, H. and Rojas, E. (1996) Zn^{2+} interaction with Alzheimer amyloid beta protein calcium channels. *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 1710–1715.
38) Durell, S. R., Guy, H. R., Arispe, N., Rojas, E. and Pollard, H. B. (1994) Theoretical models of the ion channel structure of amyloid β-protein. *Biophys. J.*, **67**, 2137–2145.
39) Kawahara, M. and Kuroda, Y. (2000) Molecular mechanism of neurodegeneration induced by Alzheimer's β-amyloid protein: channel formation and disruption of calcium homeostasis. *Brain Res. Bull.*, **53**, 389–397.
40) Kawahara, M., Arispe, N., Kuroda, Y. and Rojas, E. (2000) Alzheimer's β-amyloid, human islet amylin and prion protein fragment evoke intracellular free-calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell-line. *J. Biol. Chem.*, **275**, 14077–14083.
41) Perutz, M. F., Finch, J. T., Berriman, J. and Lesk, A. (2002) Amyloid fibers are water-filled nanotubes. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 5591–5595.
42) Flaten, T. P. (2001) Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. *Brain Res. Bull.*, **55**, 187–196.
43) Cherny, R. A., Atwood, C. S., Xilinas, M. E., Gray, D. N., Jones, W. D., McLean, C. A., Barnham, K. J., Volitakis, I., Fraser, F. W., Kim, Y., Huang, X., Goldstein, L. E., Moir, R. D., Lim, J. T., Beyreuther, K., Zheng, H., Tanzi, R. E., Masters, C. L. and Bush, A. I. (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron*, **30**, 665–676.
44) Atamna, H., Killilea, D. W., Killilea, A. N. and Ames, B. N. (2002) Heme deficiency may be a factor in the mitochondrial and neuronal decay of aging. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 14807–14812.
45) Namekata, K., Imagawa, M., Terashi, A., Ohta, S., Oyama, F. and Ihara, Y. (1997) Association of transferrin C2 allele with late-onset Alzheimer's disease. *Hum. Genet.*, **101**, 126–129.
46) Imagawa, M., Naruse, S., Tsuji, S., Fujioka, A. and Yamaguchi, H. (1992) Coenzyme Q_{10}, iron, and vitamin B_6 in genetically-confirmed Alzheimer's disease, *Lancet*, **340**, 671.
47) Pratico, D., Uryu, K., Sung, S., Tang, S., Trojanowski, J. Q. and Lee, V. M. (2002) Aluminum modulates brain amyloidosis through oxidative stress in APP transgenic mice. *FASEB J.*, **16**, 1138–1140.
48) Kawahara, M., Kato-Negishi, M., Hosoda, R., Imamura, L., Tsuda, M. and Kuroda, Y. (2003) Brain-derived neurotrophic factor protects neurotoxixity of cultured rat hippocampal neurons induced by aluminum maltolate. *J. Inorg. Biochem.* (in press).