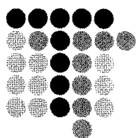

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Iron Can Accelerate the Conjugation Reaction between Abeta 1-40 Peptide and MDA

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder characterized pathologically by senile plaques, neurofibrillary tangles, and synapse loss. Especially, extracellular beta-amyloid (Abeta) deposition is a major pathological hallmark of Alzheimer's disease (AD). In AD senile plaques, high level of iron and carbonylated Abeta were detected. Iron has a Lewis acid property which can increase the electrophilicity of carbonyls, which may react catalytically with nucleophiles, such as amines. Hence, this study investigated whether or not iron could promote the carbonylation of amine with malondialdehyde (MDA) in the physiological condition. As the basic study, we examined that iron might promote the conjugation reaction between propylamine, monoamine molecule and MDA in the physiological condition. As the concentration of iron increased, the fluorescence intensity produced from the conjugation reaction increased in a dose-dependent manner. Instead of propylamine, we applied the same reaction condition to Abeta 1-40 peptide, one of major components founded in AD senile plaques for the conjugation reaction. As the result, the fluorescence intensity produced from the conjugation reaction between Abeta 1-40 peptide and MDA showed the similar trend to that of the reaction used with propylamine. This study suggests that iron can accelerate the conjugation reaction of MDA to Abeta 1-40 peptide and play an another important role in deterioration of AD brain.

Keywords: Beta-amyloid, Iron, Conjugation, Malondialdehyde, Senile plaque, Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized pathologically by senile plaques, neurofibrillary tangles, and synapse loss[1]. Especially, extracellular beta-amyloid (Abeta) deposition is a major pathological hallmark of Alzheimer's disease (AD)[2]. The 40-residue form of Abeta (Abeta1-40) along with its various amino-terminal derivatives appears to predominate in fully developed plaques[3]. Abeta peptides readily form beta-sheets structure, oligomers, protofibrils, fibrils, and subsequently co-aggregate with other proteins to produce senile plaques[4,5]. Abeta is cytotoxic and capable of triggering oxidative stress and neurodegeneration[6]. Its oligomers (i.e. 5-10 Abeta monomers) are the most toxic forms[7,8]. The physico-chemical nature of these deposits *in vivo* remains poorly understood[9].

Several mechanisms of formation of senile plaque were proposed. Factors such as pH, metal ions, cholesterol, reactive oxygen species, and ApoE4 have been shown to influence the Abeta aggregation process[10]. Iron-catalyzed oxidation accelerates Abeta aggregation *in vitro*[11]. Regional accumulation of aluminum, zinc, or other polyvalent cations may accelerate Abeta aggregation and senile plaque formation through electrostatic cross-linking of Abeta monomers independent of redox chemistry. In addition, Abeta aggregation is greatly facilitated in vitro by low concentration of either aluminum or zinc[12,13]. Meanwhile, *in vitro*, aldehydes generated from different sources were able to enhance Abeta aggregation at every stage[14].

However, the mechanisms of formation of Abeta deposits in senile plaque of AD brain, are not clear yet. The major pathological feature of AD brain is the presence of extensive oxidative stress, including elevated levels of protein carbonyls, 8-hydroxyguanine and lipid peroxidation[15]. Moreover, several studies revealed an abnormal enrichment of iron associated with insoluble Abeta plaques from post-mortem AD brains and purified senile plaque cores[16,17].

In the first place, effects of carbonyls on the AD brain are investigated. Increased levels of MDA and HNE, major components made from lipid peroxidation, were detected in the plasma of AD patients[18]. Protein carbonyls are elevated in vulnerable regions of AD brain[19-21]. Protein carbonyl content is increased 42 and 37% in the Alzheimer's hippocampus (HIP) and interior parietal lobule (IPL) regions, which contain abundant senile plaques (SPs) and neurofilamentary tangles (NFTs), respectively, relative to AD cerebellum, whereas carbonyl content in control HIP and IPL is similar to that of control cerebellum[19]. In detail, carbonylated protein adducts have been detected in the senile plaques[22,23]. Meanwhile, MDA are not only capable of enhancing the rate of formation of Abeta beta-sheets, oligomers and protofibrils but also of increasing the size of the aggregates[10]. Reactive carbonyl metabolites generated via cholesterol ozonolysis during inflammation[24] have been shown to be capable of covalently modifying and accelerating Abeta aggregation[25].

Meanwhile, Generally, it is well noted that iron increases the oxidative damages[26]. Moreover, it is known that because of its potential Lewis acidic character, iron can increase the electrophilicity of the β-carbon by the coordination with oxygen of α,β-unsaturated carbonyl compound[27]. Hence, This study had focused on the possible role of iron which may promote the carbonylation of nucleophilic amines of Abeta with α,β-unsaturated carbonyl compounds through increasing the electrophilicity of carbonyl compounds.

Therefore, this study investigated whether or not iron promote the carbonylation of propylamine as a simple monoamine molecule and Abeta with MDA in the physiological condition.

Iron Promotes the Conjugation Reaction of Propylamine with MDA Depending on Its Concentration

In nucleophilic groups of proteins, amino groups are major components. Therefore, to simplify the reactivity of proteins, propylamine having one amino group, was used for a simple reaction. Reaction solution was the neutral buffer condition as 10 mM sodium phosphate buffer (pH 7.4). As the concentration of iron ion (+3) increased from 0.01 to 1 μM, the fluorescence intensity of MDA-propylamine conjugation increased than that of the control (Con-pr) (Figure 1).

After incubation at 37°C for 12 h, the fluorescence intensity of MDA-propylamine conjugation in 1 μM of iron ion (+3) increased significantly 17.2% than that of the control (148.8±4.0% vs. 131.6±4.8%). The fluorescence intensities of MDA-propylamine conjugation were dependent significantly on the concentrations of iron ion (+3) and the reaction time.

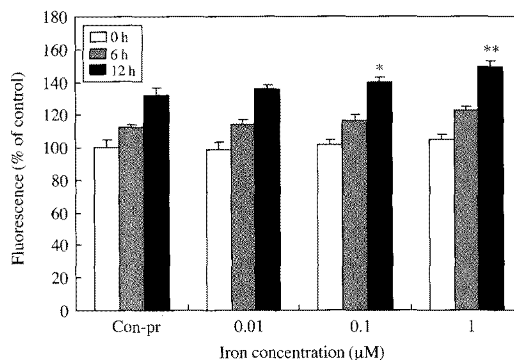

Figure 1. Effect of iron ion (+3) on MDA-propylamine conjugation. The solution of propylamine (final concentration 100 μM), was mixed with MDA,100 mM and ferric (III) chloride (0.01, 0.1 and 1.0 μM) respectively in 10 mM phosphate buffer (pH 7.4) at 37°C. The reaction solutions were incubated at 37°C for 12 hours. The error bars represent standard deviations (SDs). n=3, *$P<0.05$ and **$P<0.01$ as compared to the controls (Con-pr).

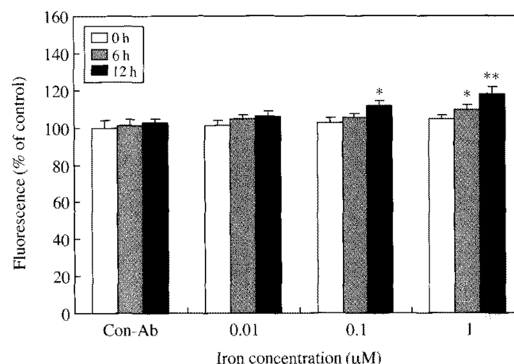

Figure 2. Effect of iron ion (+3) on MDA-Abeta 1-40 peptide conjugation in 10 mM phosphate buffer (pH 7.4) at 37°C. The solution of Abeta 1-40 peptide (final concentration 10 μM), was mixed with MDA, 100 mM and ferric (III) chloride (0.01, 0.1 and 1.0 μM) respectively in 10 mM phosphate buffer (pH 7.4) at 37°C. The reaction solutions were incubated at 37°C for 12 hours. The error bars represent standard deviations (SDs). n=3, *$P<0.05$ and **$P<0.01$ as compared to the controls (Con-Ab).

Iron Promotes the Conjugation Reaction of Abeta 1-40 with MDA Depending on Its Concentration

It is known that Abeta molecule composes are 2 lysines and 1 arginine, which are vulnerable for inter-

action with aldehydes[29]. Therefore, the reactivity of Abeta was expected to be similar to that of propylamine. We used Abeta 1-40 peptide, one of major components founded in AD senile plaques for the conjugation reaction.

As the concentration of iron ion (+3) increased from 0.01 to 1.0 μM, the fluorescence intensity of MDA-Abeta 1-40 peptide conjugation increased than that of the control (Figure 2).

After 12 h, the fluorescence intensity of MDA-Abeta 1-40 peptide conjugation in 1.0 μM of iron ion (+3) increased significantly 15.5% than that of the control (117.8±4.2% vs. 102.3±2.4%). The fluorescence intensities of MDA-Abeta 1-40 peptide conjugation were dependent significantly on the concentrations of iron ion (+3) and the reaction time, similar to those of propylamine.

Discussion

Abeta is cytotoxic and capable of triggering oxidative stress and neurodegeneration[6]. Its oligomers (i.e. 5-10 Abeta monomers) are the most toxic forms[7,8]. Therefore, to understand the detail mechanism of formation of senile plaques produced from interactions between Abeta and other biomolecules will be contributed to solving the AD.

This study had focused on interactions between Abeta, carbonyl molecule and iron which are high level in AD senile plaques. Especially, we hypothesized that iron as a Lewis acid might promote the carbonylation of Abeta with MDA. Because although under solvent free condition, coordination of iron with oxygen of α,β-unsaturated carbonyl compound increases the electrophilicity of the β-carbon[27], our hypothesis was acceptable. Moreover, it is reported that iron catalyzes similar conjugation reaction (aza-Michael reaction) between enones with carbamates[30].

This study used MDA as a representative carbonyl component which is one of major carbonyls produced in oxidative stress-induced lipid peroxidation and is detected in AD senile plaques.

As the basic study, we examined that iron might promote the conjugation reaction between propylamine, monoamine molecule and MDA in the physiological condition. As the concentration of iron increased, the fluorescence intensity produced from the conjugation reaction increased in a dose-dependent manner.

In the fluorescence analysis for the MDA-modification of amine, fluorescence intensity with excitation maximum at 387 nm and emission maximum at 455 nm means the formation of 1,4-dihydropyridine-3,5-dicarbaldehyde derivative of major adducts produced from conjugation reaction between amine and MDA[31].

In addition to propylamine, we used Abeta 1-40 peptide, one of major components founded in AD senile plaques for the conjugation reaction. It is known that Abeta molecule composes are 2 lysines and 1 arginine, which are vulnerable for interaction with formaldehyde and other aldehydes[29]. As the concentration of iron increased, the fluorescence intensities produced from the conjugation reaction improved in a dose-dependent manner similar to that of the reaction conducted with propylamine. Especially, as shown in Figure 1 and 2, MDA-modification of propylamine and Abeta 1-40 peptide in 1 μM of iron increased significantly after 12 h reaction.

Thus far, it is well known that in AD brain, iron induces oxidative modification of Abeta through the oxidative stress[32]. However, this study demonstrates for the first time the new role of iron which can catalyze the conjugation reaction of Abeta with MDA which is the major adduct of lipid peroxidation. Therefore, it is strongly expected that iron may accelerate the modification, fibrillization and then aggregation of Abeta through catalyzing the carbonylation of Abeta with MDA in AD brain.

In conclusion, in the physiological condition, iron could promote the conjugation reaction between amine and MDA and between Abeta 1-40 peptide and MDA. As the carbonyls can improve inter- and/or intra- cross-link reaction of the protein[10], iron may accelerate the formation of senile plaque in AD brain.

Materials & Methods

Reagents and Apparatus

Abeta 1-40 TFA peptide was purchased from rPeptide (Athens, Georgia, USA). Propylamine, 1,1,3,3-tetramethoxypropane and other reagents were obtained from Sigma-Aldrich Co. (Saint Louis, MO, USA). Fluorescence spectra were recorded with a Perkin-Elmer LS 55 spectrometer (Waltham, Massachusetts, USA).

Preparation of Malondialdehyde

Malondialdehyde (MDA) was prepared by hydrolysis of 1,1,3,3-tetramethoxy-propane with 1 M HCl at 37°C for 30 min, neutralized with 1 M NaOH, and the concentration was determined from the extinction coefficient at 245 nm of 14,700 mol^{-1} cm^{-1} as previously described[28].

Conjugation Reaction of Between MDA and Propylamine or Abeta 1-40 Peptide Catalyzed by Iron Ion (+3)

The solution of propylamine, 100 μM or Abeta 1-

40 peptide,10 μM, was mixed with malondialdehyde (MDA), 100 mM and ferric (III) chloride (0.01, 0.1, 1.0 μM) respectively in 10 mM phosphate buffer (pH 7.4) at 37°C. The reaction solutions were incubated at 37°C and taken at 0, 6 and 12 hours for analysis of content of MDA-propylamine and MDA- Abeta 1-40 peptide conjugation.

Measurement of MDA-propylamine and MDA- Abeta 1-40 Peptide Conjugation

Quantitation of the content of MDA-propylamine and MDA- Abeta 1-40 peptide conjugation were done at 0, 6 and 12 hours by fluorescence spectrometer at excitation wavelength 387 nm and emission wavelength 455 nm.

Statistical Analysis

The means and standard deviations were calculated for all experiments. The data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test to determine whether means were significantly different from the control. In all cases, *, P-value of <0.05 and **, P value of <0.01 were used to determine the significance.

Acknowledgements

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References

1. Katzman, R. & Saitoh, T. Advances in Alzheimer's disease. *FASEB J* **5**:278-286 (1991).
2. Selkoe, D. J. Alzheimer's disease: genes, protein and therapy. *Physiol Rev* **81**:741-766 (2002).
3. Mori, H. *et al.* Mass spectrometry of purified amyloid beta protein in Alzheimer's disease. *J Biol Chem* **267**: 17082-17086 (1992).
4. Stanyer, L., Betteridge, D. J. & Smith, C. C. Exaggerated polymerization of beta-amyloid 40 stimulated by plasma lipoproteins results in fibrillar Abeta preparations that are ineffective in promoting ADP-induced platelet aggregation. *Biochim Biophys Acta* **1674**:305-311 (2004).
5. Ma, J. *et al.* Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* **372**: 92-94 (1994).
6. Loo, D. T. *et al.* Apoptosis is induced by beta-amyloid in cultured central nervous system neurons. *Proc Natl Acad Sci USA* **90**:7951-7955 (1993).
7. Klein, W. L., Stine, W. B. Jr. & Teplow, D. B. Small assemblies of unmodified amyloid-protein are the proximate neurotoxin in Alzheimer's disease. *Neurobiol Aging* **25**:569-580 (2004).
8. Kayed, R. *et al.* Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**:486-489 (2003).
9. Seidler, N. W., Craig, H. D. & Squire, T. J. Endogenous plastic composite material in the Alzheimer's brain. *Med Hypotheses* **67**:467-470 (2006).
10. Chen, K., Maley, J. & Yu, P. H. Potential implications of endogenous aldehydes in beta-amyloid misfolding, oligomerization and fibrillogenesis. *Journal of Neurochemistry* **99**:1413-1424 (2006).
11. Dyrks, T. *et al.* Amyloidogenicity of βA4-bearing amyloid protein precursor fragments by metal-catalyzed oxidation. *J Biol Chem* **267**:18210-18217 (1992).
12. Mantyh, P. W. *et al.* Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of beta-amyloid peptide. *J Neurochem* **61**:1171-1174 (1993).
13. Bush, A. L. *et al.* Modulation of A/3 adhesiveness and secretase site cleavage by zinc. *J Biol Chem* **269**:12152-12158 (1994).
14. Chen, K., Kazachkov, M. & Yu, P. H. Effect of aldehydes derived from oxidative deamination and oxidative stress on beta-amyloid aggregation; pathological implications to Alzheimer's disease. *J Neural Transm* **114**:835-839 (2007).
15. Butterfield, D. A., Perluigi, M. & Sultana, R. Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. *Eur J Pharmacol* **545**:39-50 (2006).
16. Lovell, M. A. *et al.* Copper, iron and zinc in Alzheimer's disease senile plaques. *J Neurol Sci* **158**:47-52 (1998).
17. Liu, G. *et al.* Metal exposure and Alzheimer's pathogenesis. *J Struct Biol* **155**:45-51 (2006).
18. Dib, M. *et al.* Can MDA be used as a biological marker of progression in neurodegenerative disease? *J Neurol* **249**:367-374 (2002).
19. Hensley, K. *et al.* Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J Neurochem* **65**:2146-2156 (1995).
20. Castegna, A. *et al.* Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radic Biol Med* **33**:562-571 (2002).
21. Castegna, A. *et al.* Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. *J Neurochem* **82**: 1524-1532 (2002).
22. Dei, R. *et al.* Lipid peroxidation and advanced glycation end products in the brain in normal aging and in Alzheimer's disease. *Acta Neuropathol (Berl)* **104**: 113-122 (2002).
23. Sayre, L. M. *et al.* 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in

Alzheimer's disease. *J Neurochem* **68**:2092-2097 (1997).
24. Wentworth, P. Jr. *et al.* Evidence for ozone formation in human atherosclerotic arteries. *Science* **302**:1053-1056 (2003).
25. Zhang, Q. *et al.* Metabolite-initiated protein misfolding may trigger Alzheimer's disease. *Proc Natl Acad Sci USA* **101**:4752-4757 (2004).
26. Sayer, L. M. *et al.* Metal ions and oxidative protein modification in neurological disease. *Ann Ist Super Sanita* **41**:143-164 (2005).
27. Chua, C. M. *et al.* The iron (III) chloride-mediated 1,4-addition of mercaptans to α,β-unsaturated ketones and esters under solvent free conditions. *Tetrahedron Letters* **47**:7375-7380 (2006).
28. Esterbauer, H., Schaur, R. J. & Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol Med* **11**:81-128 (1991).
29. Gubisne-Haberle, D. *et al.* Protein cross-linkage induced by formaldehyde derived from semicarbazide-sensitive amine oxidase-mediated deamination of methylamine. *J Pharmacol Exp Therap* **310**:1125-1132 (2004).
30. Kobayashi, S., Kakumoto, K. & Sugiura, M. Transition metal salts-catalyzed aza-Michael reactions of enones with carbamates. *Org Lett* **4**:1319-1322 (2002).
31. Yamada, S. *et al.* Immunochemical detection of a lipofuscin-like fluorophore derived from malondialdehyde and lysine. *J Lipid Res* **42**:1187-1196 (2001).
32. Sayre, L. M., Moreira, P. I., Smith, M. A. & Perry, G. Metal ions and oxidative protein modification in neurological disease. *Ann Ist Super Sanita* **41**:143-164 (2005).