Synergistic effect of N-terminal pyroglutamyl amyloid ß protein in Alzheimer' s disease and in normal aging

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Abstract

Amyloid ß protein (Aß) has been considered as the main pathogenetic basis of Alzheimer' s disease (AD). Substantial evidence indicates that the soluble Aß aggregates containing N-terminally truncated Aß starting with pyroglutamate at position 3 ($Aß_{PE3}$) and position 11($Aß_{PE11}$) account for the major neuronal toxicity of AD. In addition to the heterogeneity in soluble Aß aggregate, the composition ratio of Aß variants in the brain from AD and in normal aging possess a significant role for the development of AD. For this reason, we postulate that Aß variants with different composition ratio may cause aggregation behavior entirely different. In this study, two mixtures, AD and NA, composed of three Aß variants ($Aß_{1-40}$, $Aß_{PE3-40}$, $Aß_{PE11-40}$) with different composition ratio were investigated. Thioflavine T fluorimetric assay revealed that AD mixture with a high $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio has highly increased ß-sheet structure compared with the three individual Aß variants. By contrast, NA mixture with a low $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio leads to an unobvious increase. This suggests that $Aß_{PE3-40}$ may have synergistic effect to regulate the aggregation propensities of the Aß mixtures. Surface plasmon resonance kinetics assay demonstrated that the aggregation rates of the three soluble Aß variants interacting both AD and NA mixtures have a consistent of order as follows, $Aß_{PE3-40}$> $Aß_{PE11-40}$> $Aß_{1-40}$. Both $Aß_{PE3-40}$ and $Aß_{PE11-40}$ have a higher aggregation rate than $Aß_{1-40}$ to form aggregates. Therefore, the investigated N-terminal pyroglutamyl Aß variants and their composition ratio in mixtures may play an important role to regulate aggregation behaviors and to influence the development of AD. [Life Science Journal. 2009; 6(3): 80– 85] (ISSN: 1097 – 8135).

Key words: Alzheimer' s disease, amyloid, surface plasma resonance, synergistic effect, amyloid ß protein (Aß), amyloid ß precursor protein (AßPP), Alzheimer' s disease (AD), normal aging (NA), surface plasmon resonance (SPR), thioflavine T (ThT)

1 Introduction

Alzheimer' s disease (AD), a neurodegenerative disease, is the most common cause of dementia in the elderly population. This widespread progressive neurodegeneration characterized by the presence of proteinaceous deposits in the brain is described as amyloid. The extracellular deposition of amyloid ß protein (Aß) and the intracellular generation of neurofbrillary tangles are the main histopathological features of AD (1,2).

Aß is a 39- to 43-amino acid polypeptide, and is a normal metabolic product which can be found in cerebrospinal fluid and plasma (3). Aß is derived from the proteolytic product of amyloid ß precursor protein (AßPP) through the cleavage of ß-secretase and ?-secretase (4,5). Authentic evidence indicates that several factors can lead to the formation of amyloid plaques in AD (2) including (i) genetic mutations of APP resulting in early-onset familial AD (FAD), and the over expression of APP resulting from elevated gene dosage in trisomy 21 (Down' s syndrome), (ii) FAD-causing mutations on chromosome 14 and 1 in genes encoding the homologous presenilin proteins PS1 and PS2, which affect APP processing, (iii) apolipoprotein E4 allele which lower the average age of AD. These factors can result in two predominant aggregates of Aß including $Aß_{1-40}$ and $Aß_{1-42}$ which are the primary component in senile plaques (6,7).

Although previous studies demonstrate fibrillar form of Aß is inferred as a key role leading to the pathogenesis of AD. Recent data show that the more neurotoxic forms of Aß are small, still water-soluble oligomers, amyloid-derived diffusible lignds (8) and protofibrils (9) which correspond better than fibrils with neurodegeneration. In addition to $Aß_{1-40}$ and $Aß_{1-42}$, N-terminal truncated forms of water soluble Aß were also seen in Aß plaques of the brain of AD and Dome syndrome patients. The most common forms of N-terminally truncated Aß is post-translationally modified N-terminal pyroglutamyl Aß variants, termed $Aß_{PE3-40/42}$, $Aß_{PE11-40/42}$ and p3 ($Aß_{17-40/42}$) (10,11). The C-terminal heterogeneity of Aß and its role in the pathogenesis of AD have been well characterized (2,12). Several studies demonstrated that N-terminal pyroglutamyl Aß variants, $Aß_{PE3-40/42}$ and $Aß_{PE11-40/42}$, can stabilize the peptides against degradation and they appear very early in the disease progress to show an enhanced cytotoxicity (13,14).

Most recent investigation show that the molecular composition ratio of water-soluble Aß variants in the

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soluble Aß aggregates between AD patients and normal aging (NA) individuals is unlike; the major differentiation is the molecular composition ratio of N-terminal pyroglutamyl Aß variants in aggregates which can make different depositability and cytotoxicity for the development of AD (15). In this study, the mixtures of three Aß variants, including two pyroglutamyl Aß variants ($Aß_{PE3-40}$ and $Aß_{PE11-40}$) and a full-length $Aß_{1-40}$ at different molecular composition ratios, were investigated to study the variations of aggregation propensities induced by composition change

2. Materials and Methods

All solvents and chemical used were either of analytical grade or chemically pure. Aß peptides, including $Aß_{1-40}$, $Aß_{PE3-40}$ and $Aß_{PE11-40}$, were purchased from AnaSpec (San Jose, CA). Thioflavine T (ThT), dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS) were obtained from Sigma Chemical (St. Louis, MO). All of the surface plasmon resonance (SPR) experiments used in kinetics assay of Aß variants aggregation were performed on a Biacore X apparatus, at 25 °C. The instrument, sensor chips (type CM5), and coupling reagents, including (N-ethyl-N'- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and ethanolamine HCl), were from Biacore AB (Uppsala, Sweden).

Preparation of soluble Aß Solutions.

Prior to analysis, the lyophilized amyloid peptides were subjected to a disaggregation procedure described by Dahlgren et al. (16). Afterward, stock solutions of $Aß_{1-40}$, $Aß_{PE3-40}$ and $Aß_{PE11-40}$ in a concentration of 1 mM were prepared in pure DMSO. Aß solutions treated in this way have been described to be free of oligomeric species (17,18).

Two soluble Aß variants mixtures at the molecular composition ratios referring to the investigation on AD and NA individuals described by Piccini et al. (15) with little modifications, AD ($Aß_{1-40}$, 36%; $Aß_{PE3-40}$, 48%; $Aß_{PE11-40}$, 16%) and NA ($Aß_{1-40}$, 40%; $Aß_{PE3-40}$, 29%; $Aß_{PE11-40}$, 31%), and three soluble Aß variants were suspended in PBS and kept for 24h at room temperature, at a final concentration of 1μM, PH 7.2, for subsequent analyses.

SPR Kinetics Binding Assay

SPR biosensing technology has been chosen as analytical tool to study ligand-ligate binding kinetics, which is capable of the ability to detect specific binding events between target biomolecules in liquid phase (ligate) and a specific binding partner immobilized on chip surface (ligand) without the use of labeling molecules on the target molecules and tedious processing procedures keeping peptides in native state.

In this study, SPR biosensor was adopted to investigate the real-time aggregation kinetics of the two Aß mixtures and the three Aß variants in detail. The three Aß variants kept for 24h at room temperature were separately immobilized onto chip surface as ligand by using standard amine coupling method (19). Sensor chips were first activated with an injection of a 1:1 ratio of 0.4M EDC and 0.1M NHS at a flow rate of 20μL/min for 7min. The three Aß variants, at 10μM in 10 mM sodium acetate, pH 4.0, were injected over the activated surface for 7 min. The remaining activated surface groups were blocked with a 7-min injection of 1M ethanolamine, pH 8.0. The SPR signals from each of the Aß variants result in 500-800 Biacore response units (RU).

These three immobilized Aß variants were then used to interact with the incubated soluble Aß variants and the two incubated soluble Aß mixtures. The binding data were analyzed using the BIA evaluation program.

Thioflavine T Binding Assay

The three Aß peptides and two mixtures, AD and NA, were aggregated in 100 μl of RPMI buffers, at a concentration of 100nM, for 24h at room temperature. Ten μl of each reaction mixture were mixed with 990μl of ThT (3 μM in 50 mM sodium phosphate, pH 6.0), and the fluorescence was subsequently measured at Ex/Em of 450/482 nm by a fluorescence spectrophotometer (Hitachi F-4500). The relative fluorescence intensity was defined by taking fluorescence of 100 nM $Aß_{1-40}$ aggregated for 24 h as 100 %.

3. Results

Thioflavine T binding to amyloid is a specific interaction for anti-parallel ß-pleated sheet secondary structure which produces a change in the emission spectrum of ThT (20). Thereby, the emission intensity of ThT is proportional to the total quantity of ß-pleated sheet amyloid. Fig. 1 shows that after a 24h of incubation time, $Aß_{PE11-40}$ revealed a highest amount of ß-pleated sheet amyloid among the three tested Aß variants and AD mixture displayed a much higher amount of ß-pleated sheet amyloid than does NA mixture. In our experiments, both two mixtures have a close composition ratio of $Aß_{1-40}$, but AD mixture having a high $Aß_{PE3-40}/Aß_{PE11-40}$ (48:16) composition ratio revealed a much higher increase in the amount of ß-pleated sheet amyloid than the three tested Aß variants under the same test condition of peptide concentration. By contrast, the NA mixture, which has a low $Aß_{PE3-40}/Aß_{PE11-40}$ (29:31) composition ratio, leads to a less amount of ß-pleated sheet amyloid than does AD mixture. The amount of ß-pleated sheet amyloid of NA mixture is only a little higher than does $Aß_{1-40}$.

To measure the aggregation propensities of the three individual Aß variants, SPR biosensing technique was used to directly detect specific biomolecular interactions in real time through a molecular recognition mechanism (21) which is a noninvasive optical method better than the traditional approaches for measuring aggregation kinetics (22). In Fig. 2, the sensogram, showing real-time aggregation kinetics of the three individual soluble Aß variants, revealed that the order of aggregation rates was as follows, $Aß_{PE11-40} > Aß_{PE3-40} > Aß_{1-40}$. The time response of the two pyroglutamyl Aß variants showed that $Aß_{PE3-40}$ and $Aß_{PE11-40}$ are capable of much higher aggregation rate than does $Aß_{1-40}$.

Meanwhile, the aggregation propensities of both AD and NA mixtures with the three individual Aß variants were measured. In Fig. 3a, the three immobilized Aß variants interacting with AD mixture shows that $Aß_{PE3-40}$ has a highest aggregation rate and $Aß_{PE11-40}$ has a

relatively lower aggregation rate. In Fig. 3b, the three immobilized Aß variants interacting with NA mixture show that $Aß_{PE3-40}$ has a highest aggregation rate, but this time response just a little higher than does $Aß_{PE11-40}$. Both two mixtures, AD and NA, revealed a lowest aggregation rate with $Aß_{1-40}$.

4. Discussion

In ThT binding assay, the three studied Aß variants show that the more charges the N-terminal pyroglutamyl-containing Aß peptides lose in the N terminus, the peptides have a higher amount of ß-pleated sheet secondary structure. Thereby, $Aß_{PE11-40}$ has a highest quantity of ß-pleated sheet structure and $Aß_{1-40}$ has a least quantity of this specified structure. Since the lose of three charges for $Aß_{PE3}$ and six charge for $Aß_{PE11}$ could alter their conformational properties and make them more hydrophobic to forward amyloid formation. In addition, The N-terminal glutamic acid residues of Aß peptides develop pyroglutamyl species after post-translational modification making these peptides less susceptible to further proteolysis (23). The resistance to proteolysis of pyroglutamyl Aß peptides, $Aß_{PE3}$ and $Aß_{PE11}$, probably results in a varying degree of accumulation relative to other N-terminally truncated pyroglutamyl Aß showing in neuritic plaques and in diffuse plaques. However, AD mixture in ThT binding assay containing a high $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio revealed a much higher quantity of ß-pleated sheet structure. This is even higher than does $Aß_{PE11-40}$ alone. The enhanced aggregation mechanism is not clear; one possible interpretation is that $Aß_{PE3-40}$ in AD mixture may be capable of a positive synergistic effect in promoting turnover of conformational change. By contrast, NA mixture containing a low $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio shows a low quantity of ß-pleated sheet secondary structure by comparing with the three tested Aß variants. This is even less than does $Aß_{PE3-40}$ alone. In contrast to AD mixture, the role of $Aß_{PE3-40}$ in NA mixture could be a negative synergistic effect to prohibit the formation of amyloid. The aggregation propensity of pyroglutamyl-containing Aß peptides is mainly due to a stabilized formation of ß-pleated sheet secondary structure (13), however, the composition ratio should be taken into account. In this study, the ThT fluorescence binding assay demonstrated that the two pyroglutamyl-containing Aß variants have relatively higher amount of ß-pleated sheet amyloid than $Aß_{1-40}$. In addition, by varying the composition ratio of pyroglutamyl Aß variants in the tested mixtures can produce different synergistic effects to change the depositability of Aß mixtures.

Previous ThT binding assay is used to differentiate the quantity of ß-pleated sheet secondary structure of the three Aß variants. It can be used to interpret the enhancement in conformational transition by the composition ratio of the composed three Aß variants in the tested mixtures. In order to provide the binding kinetics of AD and NA mixtures with the three studied Aß variants, SPR kinetics assay was analyzed which can illustrate the differentiation in aggregation behaviors of the three Aß variants with AD and NA mixtures.

SPR kinetics assay displayed that the order of aggregation rates of the three Aß variants is correspond to the quantity of ß-pleated sheet structure of the Aß variants. This suggests that intra- and intermolecular interactions between hydrophobic parts of the Aß sequence leads to the formation of Aß aggregates. The peptide by lose of charge repulsion and stabilized ß sheet structure can obviously enhance aggregation rate (24). However, this aggregation behavior cannot be applied directly to the tested mixtures. Among the three tested Aß variants, both AD and NA mixtures have a highest aggregation rate with $Aß_{PE3-40}$, not the more hydrophobic $Aß_{PE11-40}$. In addition, AD mixture has a much higher aggregation rate with $Aß_{PE3-40}$ than with $Aß_{PE11-40}$. This may explain that AD mixture has a high $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio. By contrast, NA mixture shows a similar aggregation rate with both $Aß_{PE3-40}$ and $Aß_{PE11-40}$. This may explain that NA mixture has a low $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio.

In this study, we found that the elevated $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio can provide positive synergistic effect for the formation of ß–pleated sheet secondary structure and both two mixtures have highest aggregation rate with A $_{PE3-40}$. These results suggest that higher composition of $Aß_{PE3-40}$ can form more amyloidogenic structure and higher affinity to aggregate with pathogenic Aß mixture. $Aß_{1-40}$ has less two hydrophobic C-terminal alanine and isoleucine residues than full-length $Aß_{1-42}$ resulting in a lower aggregation propensity. A pronounced elevation of only $Aß_{1-40}$ does not lead to plaque formation but can actually really retard the deposition of $Aß_{1-42}$ in the brain (25). If $Aß_{1-40}$ is mixed with a high $Aß_{PE3-40}/Aß_{PE11-40}$ composition ratio, that can result in larger pathogenic plaques. Therefore, an adequate control on the pyroglutamyl-containing Aß variants and the composition ratio can be used to define therapeutic strategy of AD.

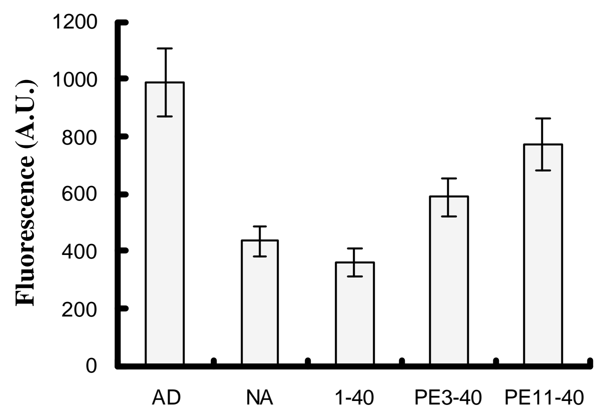


Fig. 1. Thioflavine T binding assay of $Aß_{1-40}$, $Aß_{PE3-40}$, $Aß_{PE11-40}$, and two mixtures, MD and MA. Data are expressed as fluorescence intensity in arbitrary unit as mean values ± S.D. measured from three experiments.

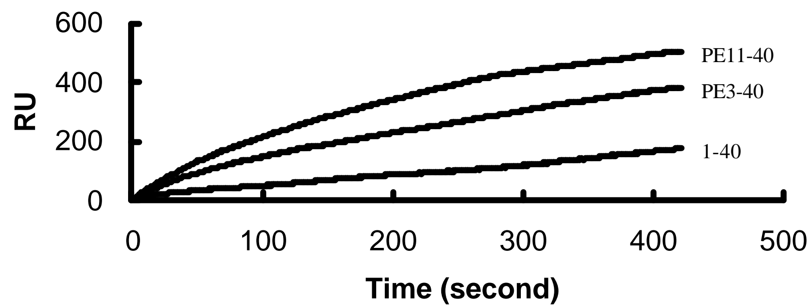
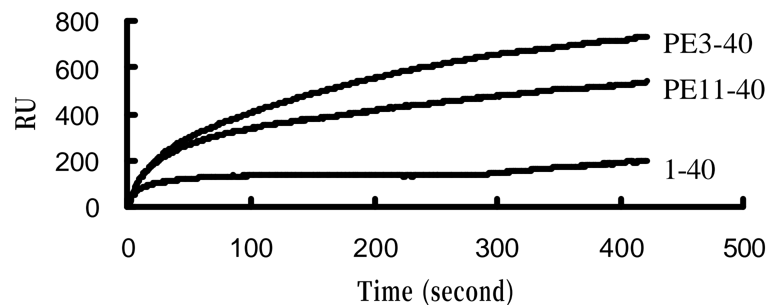
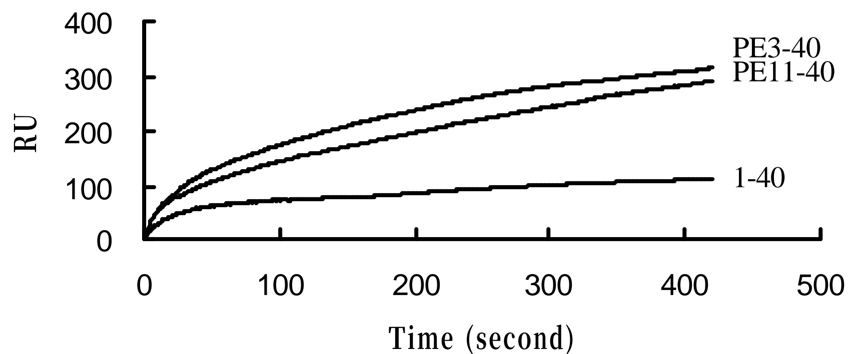


Fig.2. SPR analysis of the aggregational kinetics of $A\beta_{1-40}$, $A\beta_{PE3-40}$, and $A\beta_{PE11-40}$.After 7mins of polymerization, $A\beta_{PE11-40}$ revealed the highest aggregation rate, $A\beta_{PE3-40}$ is next, and $A\beta_{1-40}$ is lowest.



(a)



(b)

Fig.3 SPR analyses of the aggregational kinetics of mixtures (a) AD, (b) NA interact with $A\beta_{1-40}$, $A\beta_{PE3-40}$, and $A\beta_{PE11-40}$, respectively. $A\beta_{PE3-40}$ displayed a highest aggregation rate with both AD and NA mixtures. To compare with $A\beta_{PE3-40}$, $A\beta_{PE11-40}$ displayed a similar aggregation rate with NA mixture.

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