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Highly sensitive detection of amyloid-β seed by ultrasonic irradiation

超音波照射による凝集加速反応を利用した、
アミロイドβタンパク質の高感度なシード検出

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1. Introduction

It has been reported that brain tissue of Alzheimer's disease (AD) patient contains amyloid β (Aβ) aggregates, which are related to pathology of AD[1]. An Aβ monomer consists of 39 to 43 amino acid residues. This peptide aggregates over a long time in bodies to form aggregations, including oligomers and amyloid fibrils, which show the neurotoxicity[2]. It is reported that Aβ aggregates in brain appear about 20 years before the dementia symptom[3], and it is highly important to make the confirmed diagnosis in the early stage. We focus on the fact that Aβ aggregates 20 years ago before onset and aim to develop a diagnosis methodology for the presence of the aggregated nucleus (seeds), which cause neurotoxic aggregates like fibrils. When cerebrospinal fluid is added into the supersaturated monomer solution, the seeds will accelerate the aggregation reaction, allowing us to diagnose for AD.

However, it takes 5 days or longer to detect the aggregates[4], and it is not suitable for general use like medical checks. Hence, we apply ultrasonic wave to further accelerate the aggregation reaction. Recently, our group discovered that an optimum ultrasound with frequencies near 30 kHz can promote aggregation dramatically[5][6]. This phenomenon is mainly caused by the transient cavitation bubbles made by ultrasonic irradiation[7], on which hydrophobic peptides are attached and they are highly condensed and heated at the subsequent bubble collapse. We consider that this mechanism also applies to the small Aβ seeds, which are expected to exhibit hydrophobic regions. The formation of neurotoxic amyloid fibrils were evaluated by thioflavin T (ThT), which specifically binds the cross-β structure of the amyloid fibril[8].

2. Experimental procedure

For preparing the ThT solution, the ThT powder was dissolved with 50 mM glycine-NaOH buffer (pH 8.5) solution containing 100 mM NaOH to obtain the final 1 mM ThT solution.

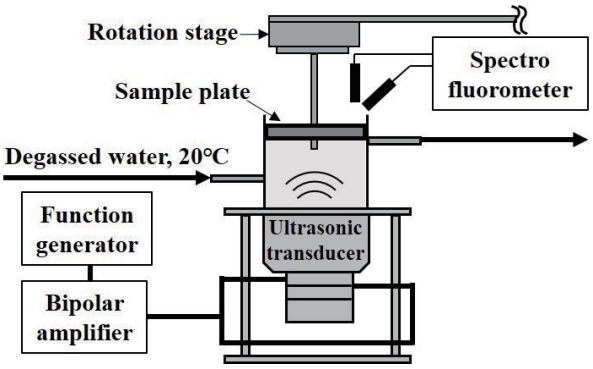

Fig. 1 Schematic of experimental system.

The lyophilized $A\beta_{1\text{-}40}$ was purchased from Peptide Institute (Lot number: 4307-v). First, the powder $A\beta_{1\text{-}40}$ was dissolved by dimethyl sulfoxide (DMSO) by stirring at 200 rpm for 10 min. Second, this solution was diluted to 10 μM Aβ solution by 66.7 mM phosphate buffered saline (PBS, pH 7.4) solution containing 100 mM NaCl. The volume fraction of DMSO:PBS was 1:19. This solution was prepared as the original Aβ solution. We made monomer solution and seed solution from this original Aβ solution.

For preparing the $A\beta_{1\text{-}40}$ monomer solution, the ThT solution was added to the original Aβ solution to obtain the final $A\beta_{1\text{-}40}$ concentration of 10 μM with stirring at 200 rpm for 5 min. This solution was then stored at -40 ℃ just before the experiments. Before use, this solution was diluted by 2-fold with DMSO and PBS. Finally, the volume fraction of DMSO:PBS was 1:4, the ThT concentration was 5 μM, and the $A\beta_{1\text{-}40}$ monomer concentration was 5 μM.

For preparing the seed solution as model target, the original Aβ solution was stirred for 48 h. Then, ultrasonic irradiation was performed for 5 h to form the aggregations with the cross-β structures. We confirmed the formation of the cross-β aggregates by the fluorescence at 483 nm (ThT emits 483 fluorescence with 450 nm excitation

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when it binds to the cross-β structure.). This solution was frozen at -40 ℃ just before the experiments. Before use, this solution was dissolved with PBS solution to an intended seed concentration.

We developed the experimental system for ultrasonic irradiation as shown in **Fig.1**. The Langevin type ultrasonic transducer with fundamental frequency of 30 kHz was tightly fixed on the bottom face of the stainless-steel cylinder with a screw. The reaction cylinder was filled with degassed water for avoiding loss of the acoustic energy caused by cavitation bubbles there. Degassed-water temperature was kept at 20 ℃. The sinusoidal voltage generated by the function generator was amplified and input to the ultrasonic transducer. The sequence of 5-min ultrasonic irradiation and 2-min incubation was repeated.

The self-made sample plate was used for the multichannel experiment. This sample plate has 12 wells. The $A\beta_{1\text{-}40}$ monomer solution and $A\beta_{1\text{-}40}$ containing the seed solution were poured into 6 channels each and sealed with parafilm. This sample plate was rotated by a rotation stage to make the ultrasonic irradiation uniformly. The rotation speed was 1/6 Hz, and we measured the fluorescence intensity every 15 min using the bundle fiber type spectrofluorometer (Excitation light: 450 nm, Emission light: 483 nm).

3. Results and Discussion

Changes in the ThT fluorescence intensity in the wells of $A\beta_{1\text{-}40}$ monomer only solution and monomer solution including the 10 nM seed solution are shown in **Fig. 2**. We normalized the fluorescence value corresponding to the maximum value. T_{half}, the time when the normalized ThT fluorescence level becomes 0.5, is an important indication to evaluate the acceleration degree. In order to reduce the measurement errors, we excluded extreme data, showing the maximum and minimum T_{half} values. Fig. 2 plots the averages of 4 data. The T_{half} values of 10 nM seed solution and monomer are 36.3 min and 63.4 min, respectively. **Fig.3** shows average and standard deviation of T_{half} of this experiment. From this result, it is clear that seed solution and monomer solution could be distinguished within ~2 h. The Aβ concentration in human cerebrospinal fluid ranges 1 ~ 6 nM [9]. Because the seed concentration of 10 nM in the present study is evaluated from the original monomer concentration, the actual seed concentration is expected to be much to lower. For example, if a single seed is composed of 100 monomers, the seed concentration will be 0.1 nM. Thus, our result suggests importance in the practical diagnosis.

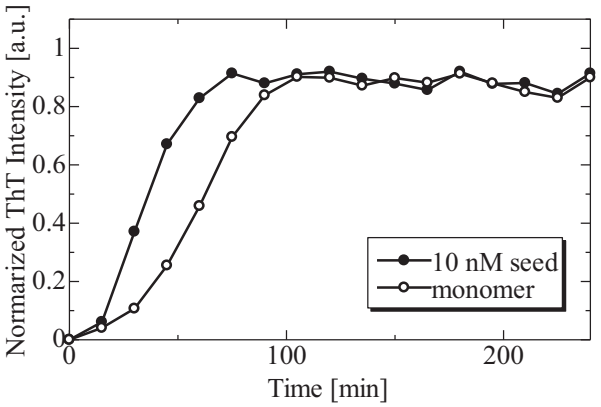

Fig. 2 Differences of aggregation between 10 nM seed solution and monomer solution. 10 nM seed solution mean 5 μM $A\beta_{1\text{-}40}$ monomer solution including $A\beta_{1\text{-}40}$ 10 nM seed.

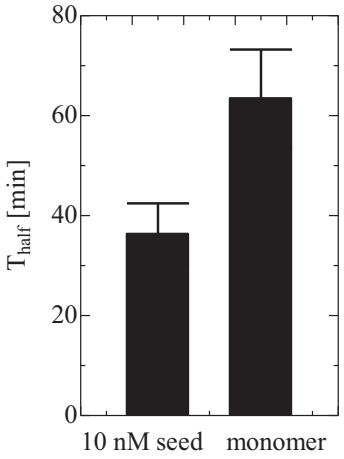

Fig. 3 Average and standard deviation of T_{half} excluding extreme dates.

4. Conclusion

We developed the seeding reaction acceleration system with ultrasonic irradiation, and demonstrated that ultrasonication can significantly accelerate the seeding reaction. This method will be useful for the diagnosis method of AD.

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