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Acoustic-Pressure Dependence of Ultrasonically-Induced Aggregation Behavior of Amyloid β Peptides

超音波照射によるアミロイド β ペプチドの異常凝集の音圧依存性

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1. Introduction

Alzheimer's disease (AD) is a kind of neurodegenerative diseases developed with age, and it is the most popular cause of dementia. The cognitive faculty of AD patients progressively decreases. The number of AD patients grows significantly because of the aging society. It is widely recognized that aggregation and deposition of amyloid β peptides (Aβ) within brain cause AD. The senile plaque, a major pathological hallmark of AD, consists of Aβ aggregates. Aβ is produced by cleavage of amyloid precursor protein (APP) by two proteases, known as β− and γ−secretases. Aβ consists of ~40 amino acid residues, and their molecular weights are about 4 kDa [1]. Aβs form fibrillar aggregates, called amyloid fibrils. However, because the aggregation activity of Aβ is not so high, the pathogenic mechanism of AD is still unclear, and the efficient therapeutic drugs or treatment approaches to AD have not been achieved. Therefore, it is important to develop a methodology to accelerate the aggregation rate of Aβ. Moreover, from the point of view of prevention or delaying progression of the disease, it has been an important issue to detect the aggrgation behavior in the early stage.

Recently, Goto *et al.* have discovered the ultrasonically-induced aggregation behavor of β_2−microglobulin (β2−m) [2, 3]. Ultrasonication to the solutions containing monomer peptides significantly accelerated fibril formation. Therefore, we focus our attention on ultrasonication for accelerating aggregation rate of Aβ.

However, its mechanism of the acceleration of the fibril formation remains unclear. For identifying the mechanism, it is very effective to investigate the pressure dependence of the phenomenon. Here, we study the acoustic-pressure dependence of ultrasonically-induced aggregation behavior of Aβ. We used 200 kHz ultrasonic tranducer in the experiment and thioflavin T (ThT) fluorescence assay for the detection of amyloid fibril.

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2. Experimental Procedure

$A\beta_{1-40}$ was used in this study. The lyophilised $A\beta_{1-40}$ was dissolved in a dimethyl sulfoxide (DMSO) and diluted by PBS (pH 7.4) to obtain the final concentration of 50 μg/ml. 500 μl of the solution was injected in the microtube and ultrasonicated for 5 h: The solution was ultrasonicated for 1 min in water and incubated for 9 min without ultrasonication. This 10-min sequence was repeated for 5 h. The temparature of water was maintained at 0 ºC.

Evaluation of $A\beta_{1-40}$ aggregates was performed by the ThT fluorescence assay. ThT was dissolved glycine-NaOH buffer (50 mM, pH 8.5) to obtain the final concentration of 5 μM. The solution was stocked at 4 ºC before use. The assay was performed every 30 min by mixing a 5-μl ultrasonicated $A\beta_{1-40}$ solution and a 50-μl ThT solution in a quartz cell. The ThT fluorescence intensity was then measured as the fluorescence wavelength at 490 nm with the excitation wavelength at 450 nm.

The morphologies of the ultrasonicated $A\beta_{1-40}$ aggregates were evaluated by an atomic-force microscopy (AFM): A 10-μl solution was dropped onto a freshly cleaved mica plate, dried for 5 min, rinsed by ultrapure water, and dried to make the substances in the solution attached on the mica plate. The tapping-mode measurement was adopted with a silicon cantilever with the stiffness of 40 N/m, showing the resonance frequency near 300 kHz. The scan frequency was 0.5 kHz.

3. Result

Fig.1 shows evolutions of the ThT fluorescence intensity of ultrasonicated (open and filled circles) and incubated (open triangle) $A\beta_{1-40}$. $A\beta_{1-40}$ was incubated without ultrasonication at room temperature to see the influence of ultrasonication. We changed the acoustic pressure by a factor of 1.6.

Fig.2 shows the AFM image of the $A\beta_{1\text{-}40}$ ultrasonicated for 5 h. We clearly observed amyloid fibrils, but failed to find them in the incubated solutions.

Fig. 3 (a) shows the wave forms detected by a needle-like transducer during the ultrasonication, and (b) and (c) are their FFT spectra.

4. Discussion

The ThT fluorescence intensity unchanged in the experiment with the low-pressure ultrasonication, but it significantly increased with a lag time near 3 h in the experiment with the high-pressure ultrasonication. Thus, we have observed the significant pressure dependence of the fibril fomation by ultrasonication.

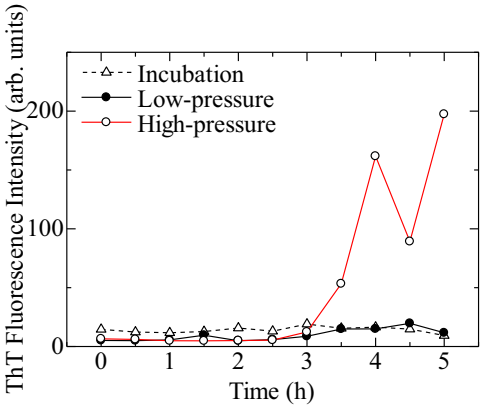

Fig.1 Change in the ThT fluorescence intensity in $A\beta_{1\text{-}40}$ solution with ultrasonication (open and filled circles) and incubation (open triangle) treatments.

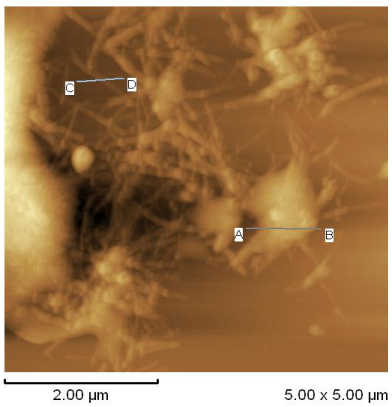

Fig.2 The AFM image of the morphology of $A\beta_{1\text{-}40}$ ultrasonicated for 5 h.

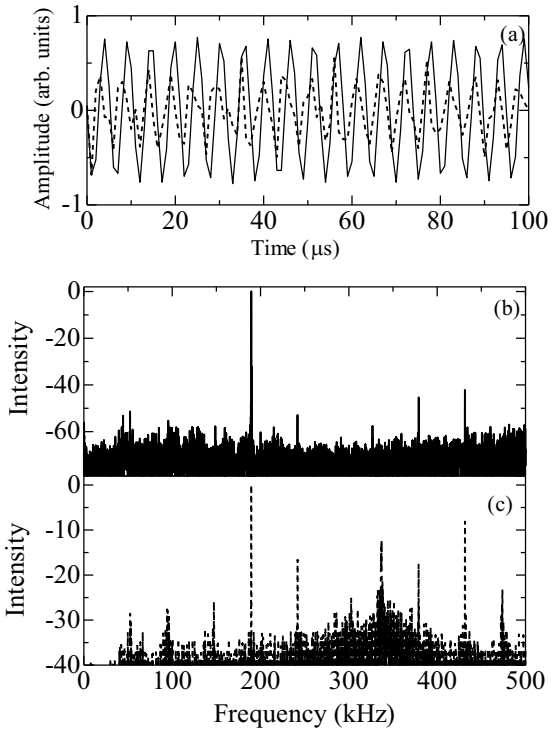

Fig.3 The wave forms and FFT spectra of high- (a, solid line, and b) and low-pressure (a, dash line, and c) ultrasound. These measurements were performed in the microtube.

In fact, each $A\beta_{1\text{-}40}$ monomers may interact with each other more strongly by higher compressive stress of the propagation of ultrasound. Furthermore, many higher harmonics are detected in the FFT spectrum of the low-pressure ultrasound, indicating larger number of cavitation bubbles. We therefore consider that the acceleration of the aggregation will be caused by structure modification by the acoustic strain field, not by the thermally activation by cavitation.

5. Conclusion

We succeeded in the promotion of fibril formation of $A\beta_{1\text{-}40}$ by ultrasonication, and showed that the aggregation activity dependended on the acoustic-pressure. We expect that this study is contibute to the initial diagnosis of AD.

References

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