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Frequency dependence of ultrasonically accelerated fibrillation phenomenon of Alzheimer-disease peptides

アルツハイマー病ペプチドの超音波による線維化加速現象の周波数依存性

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1. Introduction

Alzheimer's disease (AD), known as a major cause of dementia, remains the critical issue on the aging society. The number of AD patients is increasing yearly and it is imperative to establish an effective remedy for AD. However, the pathogenic mechanism of AD is still unclear, and hence there is no crucial cure for AD at present.

One of the primary pathological characteristics of AD is the extracellular aggregation and deposition of peptides called amyloid β (Aβ) peptides. Thus, revealing aggregation mechanism of Aβ peptides must lead to the development of treatment method for AD. Aβ peptides are proteins which consist of ~40 amino acid residues, and they are generated from the amyloid precursor protein by proteolytic activities of two proteases called β- and γ-secretases. The molecular weights of Aβs are about 4300 Da. Aβs are representative amyloidosis proteins and they form fibrillar aggregates called amyloid fibrils. These aggregates have cross-β-sheet structures where β-strands align perpendicularly to the fibril longitudinal axis.

The fact that the onset age of AD is normally higher than 60 suggests that Aβ peptides need a very long time to form the neurotoxic aggregates. Because of this characteristic of the peptides, it was difficult to clarify the pathogenesis mechanism of AD. Therefore, developing a methodology to accelerate aggregation of the peptides is required for investigating the pathogenic mechanism of AD.

Recently, Goto *et al.* showed that ultrasonic irradiation significantly accelerates the formation of amyloid fibrils for various amyloidosis proteins, including β_2-microglobulin [1] and α-synuclein [2]. Moreover, we showed that this phenomenon also occurs for Aβ peptides [3]. However, this mechanism remains unclear.

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We indicated that the cavitation bubble plays a crucial role in ultrasonically induced fibrillation phenomenon [3]. Then, we focus on the frequency dependence of this phenomenon in this study, because the frequency deeply affects the bubble dynamics. No previous report appears, studying the frequency dependence of the ultrasonically accelerated fibrillation phenomenon. Here, we study the frequency dependence of ultrasonically accelerated fibrillation phenomenon of Aβ peptides. We used $A\beta_{1\text{-}40}$ throughout this study and performed the thioflavin-T assay for evaluating the formation of amyloid fibrils of Aβ peptides. ThT selectively binds to the cross-β-sheet structure of which the fibrils are composed, and emits strong fluorescence at that moment. In other words, the fluorescence intensity of ThT increases with growth of amyloid fibrils.

2. Experiment Procedure

The laboratory-build experimental system is illustrated in **Fig. 1**. An ultrasonic transducer was attached on the bottom of a stainless-steel container. The degassed water was used to avoid power loss of ultrasonic wave by generation of bubbles, and its temperature was kept at ~10 °C by circulating cold water in the buffer tank. The degassed-cooled water was thus provided from the buffer tank.

The lyophilized Aβ was dissolved in dimethyl sulfoxide (DMSO) with stirring at 200 rpm for 10 min and diluted with phosphate buffer saline (pH 7.4) including 0.1 M NaCl to obtain the final concentration of 10 μM. The 500 μL of the Aβ solution was dispensed into the microtubes (1.5 ml vol.), which were then set in the tube holder in a circle and they were subsequently located above the transducer to perform ultrasonic irradiation for them. A single ultrasonication sequence consisted of 1-min ultrasonic irradiation and 9-min incubation. This 10-min sequence was repeated for 16 h, measuring the ThT level every 30 min.

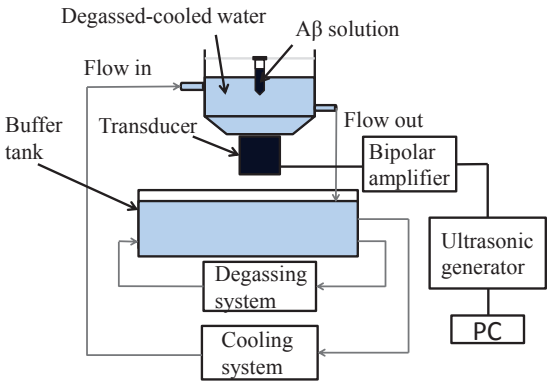

Fig. 1 Schematic of the laboratory-build experimental system for studying the frequency dependence of the ultrasonically induced fibrillation of Aβ.

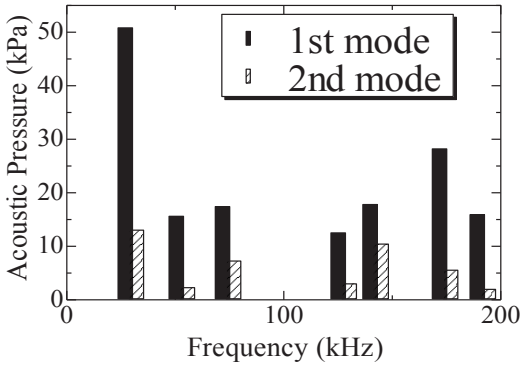

Fig. 2 Acoustic pressure of 1st and 2nd modes vs the frequency

The frequencies and acoustic pressures of ultrasonic wave were measured in individual microtubes using a 3-mm diameter handmade PZT probe. Obtained acoustic-pressure data was calibrated using a needle-type hydrophone whose sensitivity is known. **Figure 2** shows that high acoustic pressures of 1st and 2nd modes can be caused at various frequencies with this system.

The aggregation of Aβ was monitored using the ThT assay. The 1 mM ThT solution was diluted with 50 mM glycine-NaOH buffer (pH 8.5) to obtain the final concentration of 5 μM. The obtained solution was wrapped in aluminum foil and stocked at 4 °C before use. Aliquots of 5 μL were taken from the microtubes including Aβ solution and mixed with 50 μL ThT solution in a quartz cell. Subsequently, the fluorescence intensity of ThT was measured. We set an excitation wavelength at 450 nm and a range of detection wavelength at from 450 to 500 nm.

3. Result and Discussion

The relationship between aggregation kinetics and the frequency was investigated to clarify the dependence of ultrasonically accelerated aggregation phenomenon on the frequency (**Fig. 3**). The fibrillation rate showed a negative correlation with the ultrasonic frequency. This indicates that the phenomenon is dependent on the frequency and the existence of an optimal frequency for accelerating the aggregation of Aβ peptides.

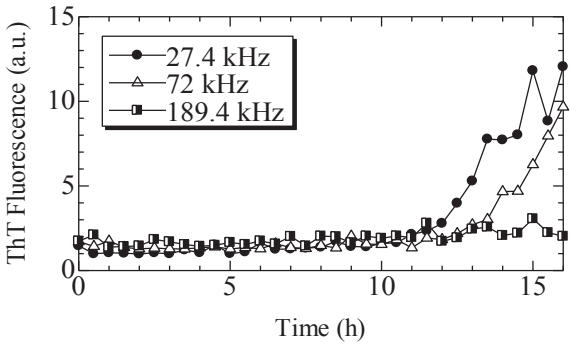

Fig. 3 Evolutions of the ThT fluorescence intensity caused by ultrasonic irradiation at the different frequencies.

Table 1 Values of acoustic pressures calibrated using a hydrophone (kPa)

	fundamental	2nd harmonics
27.4 kHz	56.7	17.8
72 kHz	13.8	2.45
189.4 kHz	18.2	3.99

The acoustic pressures in this experiment were shown in **Table 1.** Previously, it was shown that the acoustic pressure of 2nd harmonics has a positive correlation with the ultrasonically induced aggregation phenomenon [3]. However, the results display that it does not necessarily show a positive correlation with the rate of aggregation if the frequency is different. This shows that the frequency plays a more dominant role than the acoustic pressure in ultrasonically accelerated fibrillation phenomenon of Aβ peptides.

4. Conclusion

We showed that the frequency is a quite important parameter in ultrasonically accelerated fibrillation phenomenon of Aβ peptides. Furthermore, this also indicated that the cavitation bubble generated by ultrasonic irradiation is deeply related to the phenomenon.

References

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