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Influence of ultrasonic duty cycle on ultrasonically induced aggregation reaction of amyloid-β protein

アミロイドβ蛋白質の超音波誘起凝集反応に対する
超音波デューティサイクルの影響

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1. Introduction

Neurotoxic aggregates, called amyloid fibrils, originate from amyloid-β (Aβ) proteins, and they are deeply associated with the pathology of Alzheimer's disease (AD). It takes very long term, a few decades, to form amyloid fibrils, and this characteristic prevents us from understanding the aggregation mechanism in detail. There are then few effective treatments for AD. Thus, it is necessary to establish an efficient aggregation-acceleration method for Aβ proteins.

It is recently reported that aggregation reaction of several amyloidogenic proteins, including Aβ, can be drastically accelerated by the ultrasonic irradiation (UI) to the monomer solution [1]. This method allows us to fabricate amyloid fibrils shortly under physiological condition. Thus, it is expected to contribute not only to clarify the behavior of the Aβ aggregation reaction but also to the diagnosis and drug discovery for AD.

In our previous studies, we found out that an optimum ultrasonic frequency for accelerating Aβ aggregation reaction is near 30 kHz [2] and that the dominant factor affecting the aggregation reaction during UI is the transient cavitation bubbles [3]. In these aggregation experiments under UI, sample solutions were exposed to UI and then incubated for a certain time. This irradiation-incubation sequence was repeated until the aggregation reaction was completed. While the aggregation reaction mainly proceeds under UI, some related reactions may occur during incubation. Therefore, the ultrasonic duty cycle, meaning the ratio of the UI time (T_{UI}) to the incubation time (T_{inc}), will be an important factor for the aggregation reaction induced by UI. However, no study systematically investigates this.

We then investigate the relationships between the ultrasonic duty cycle and the Aβ aggregation-reaction rate accelerated by UI. We perform the UI experiment for Aβ sample solution with the optimum ultrasonic frequency, varying the UI intensity and the duty cycle. The progress of the aggregation reaction was evaluated using the thioflavin-T (ThT) fluorescence assay. ThT molecules specifically bind to amyloid fibrils and cause fluorescence, so that this method has been generally used for monitoring the aggregation reaction of Aβ proteins.

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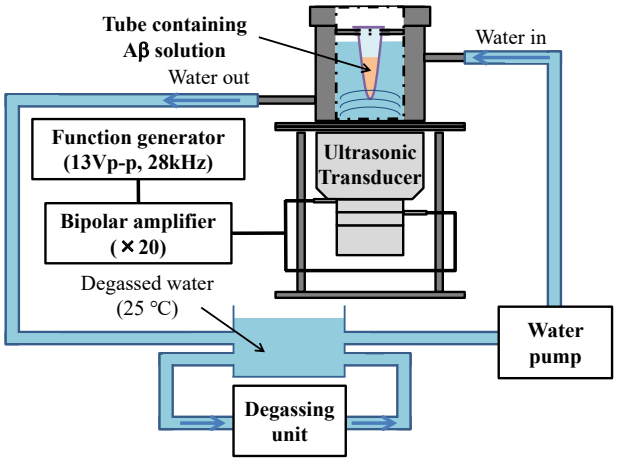

Fig. 1　Schematic of home-built experimental system for UI experiments to Aβ solution.

2. Experimental procedure

The Lyophilized-powder $Aβ_{1-40}$ protein was purchased from Peptide Institute (Lot number: 4307-v). First, the powder $Aβ_{1-40}$ was dissolved by dimethyl sulfoxide (DMSO) by stirring at 200 rpm for 10 min. Second, this solution was diluted to 5 μM by 100 mM phosphate buffer saline (PBS, pH 7.4) solution containing 100 mM NaCl. The volume fraction of DMSO and PBS was 1:19. The prepared Aβ solution was dispensed into 500 μL aliquots in each sample tube (1.5 mL). This solution was frozen at -40 °C just before an experiment. Before use, this solution was diluted by 2-fold with PBS.

For preparing the ThT solution, the ThT powder was dissolved to 5 μM by 50 mM glycine-NaOH buffer (pH 8.5) solution containing 100 mM NaOH.

We developed the experimental system for UI experiment as shown in **Fig. 1**. The Langevin-type ultrasonic transducer with fundamental frequency of 28 kHz was tightly fixed under the stainless-steel cylinder with a screw. The reaction cylinder was filled with degassed water (room temperature) for avoiding loss of the acoustic energy caused by

cavitation bubbles there. Then, the sample tube containing Aβ solution was located near the water surface of the reaction cylinder. The ultrasonic transducer was activated by burst signals generated by a function generator: T_{UI} was fixed to 30 s and T_{inc} was set at four various values between 30 and 240 s. These sequences were repeated 30 times. Every 2 times, the ThT fluorescence assay was performed: 20 μL Aβ sample solution was mixed with 20 μL ThT solution in a quartz crystal cell. We used excitation light (450 nm), and the maximum fluorescence value scanned from 440 to 500 nm was recorded as the fluorescence value.

The acoustic pressure and the temperature change in the sample tube containing the buffer solution were measured with a handmade PZT probe and radiation thermometer, respectively, before Aβ aggregation experiment. We performed an identical experiment three times to confirm the reproducibility.

3. Results and discussion

Temperature changes of the buffer solution in the sample tube caused by UI are shown in **Fig. 2**. The maximum temperatures reach nearly 35 °C; the average temperature will be different among different duty cycles.

FFT spectra measured with the PZT probe are shown in **Fig. 3**. In our previous study, we found out that aggregation reaction of Aβ induced by UI depends on the second-harmonics acoustic pressure. In this experiment, the second-harmonics intensities at several trials are large enough to accelerate Aβ aggregation reaction by comparison to the result of the previous study [2], indicating that our measurement setup proceeds the aggregation reaction.

The time courses of the ThT fluorescence intensity are shown in **Fig. 4**. Regardless of the difference in the duty cycle, the aggregation reaction of Aβ solution induced by UI appears to be depend only on T_{UI}; it is unaffected by the incubation time. This indicates that serious aggregation reaction only proceeds during UI in case of Aβ proteins, and there is little reaction progress during the incubation period.

4. Conclusion

Aggregation reaction of Aβ induced by UI does not depend on the duty cycle. And this reaction is immediately completed within 10 min in total UI time at optimum condition.

References

1. M. So *et al.*: J. Mol. Biol. **412** (2011) 568.
2. K. Nakajima *et al.*: Sci. Rep. **6** (2016) 22015.
3. K. Nakajima *et al.*: Ultrason. Sonochem. **36** (2017) 206.

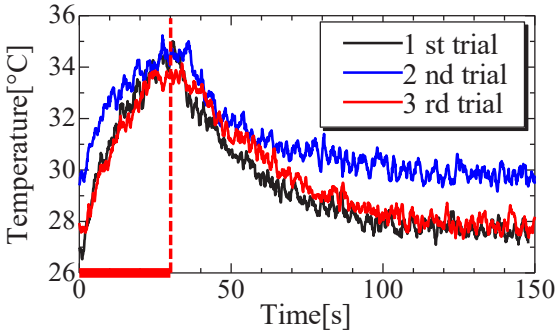

Fig. 2 Temperature changes of buffer solution in the sample tube at the several trials. The thick red line up to 30 s shows the UI period.

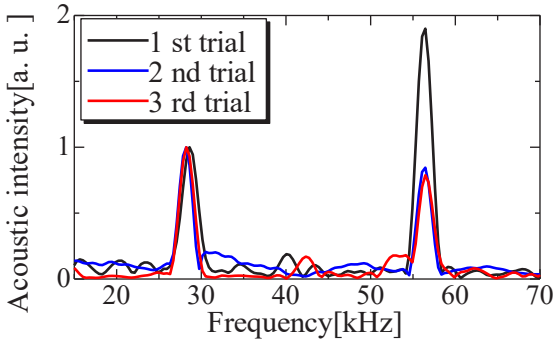

Fig. 3 Measured FFT spectra. 28 kHz (fundamental frequency) and 56 kHz (second harmonics) components appear several trials. The vertical axis is normalized by the fundamental peak value.

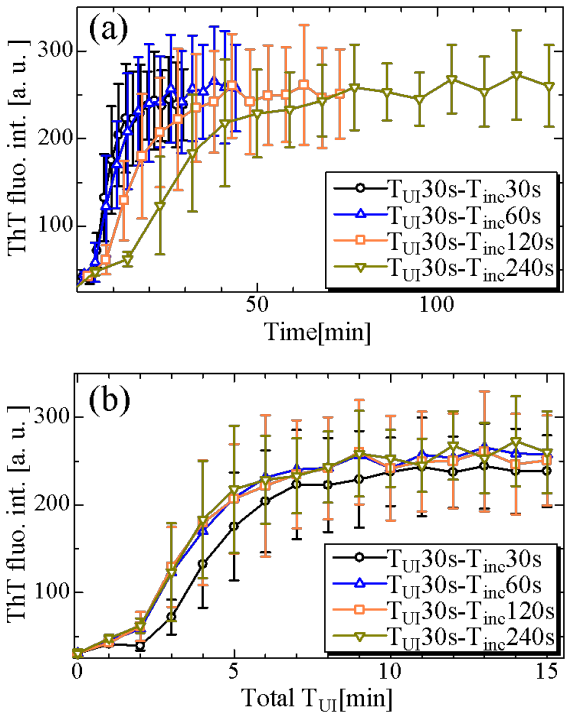

Fig. 4 Time courses of ThT fluorescence intensity of Aβ samples caused by UI with several duty cycles. The horizontal axis indicates (a) the total experimental time (total T_{UI} + total T_{inc}), (b) the total T_{UI}.