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Influence of ultrasonic irradiation condition on aggregation reaction of insulin

インスリンの凝集反応に対する超音波照射条件の影響

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1. Introduction

Amyloid fibril is an aggregate composed of protein molecules and often shows neurotoxicity, resulting in serious diseases called amyloidosis, including Alzheimer's disease. These diseases are the critical issue on the aging society, but there are few effective treatments because the formation of amyloid fibrils takes very long term, a few decades, preventing us from understanding its characteristics. Bateman and coworkers indicate that, in the case of Alzheimer's disease, amyloid β (Aβ) begins to accumulate on the cerebral cortex of patients before ~20 years of appearance of the clinical symptoms of dementia [1], suggesting that risk diagnosis in the early stage is necessary. For the early diagnosis, efficient aggregation-acceleration methods will significantly contributes to reproduce the behavior of the aggregation reaction.

It has been reported that formation of amyloid fibril is drastically accelerated by ultrasonic irradiation to monomer solutions [2]. In our previous study [3], we experimentally found an optimum frequency for accelerating Aβ aggregation to be near 30 kHz. We also suggested the ultrasonic cavitation bubble as the accelerator. Further mechanism clarification is necessary to apply ultrasonic irradiation to the diagnosis. However, Aβ is unsuitable to clarify the acceleration mechanism, because a few effects is possible to promote the reaction, not only the cavitation bubble but also temperature increase of bulk solution and the ultrasonic stirring effect. Although these contributions should be independently evaluated, it is difficult owing to higher aggregation tendency of Aβ monomers.

We then use insulin as a model protein for investigating the mechanism. Insulin is very stable and difficult to cause fibrillation under physiological condition; it can aggregate only under a specific condition such as high temperatures and acidic condition. This character is suitable to separate off some factors related to the aggregation acceleration. We here perform ultrasonic irradiation experiments to insulin solution with the optimum frequency of 26 kHz under the various irradiation conditions and investigated relationships between acoustic and temperature fields, and the aggregation reaction.

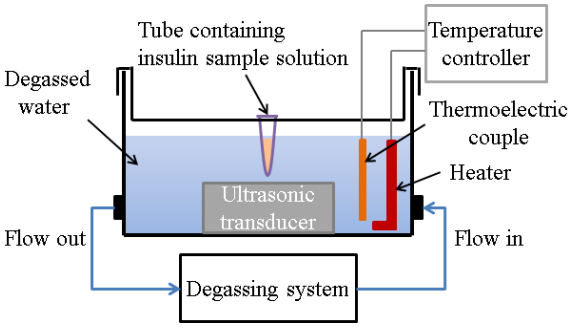

Fig. 1　Schematic of home-built experimental system for ultrasonic irradiation experiments.

2. Experimental procedure

The lyophilized powder insulin from bovine pancreas (5733.49 Da) was purchased from Sigma Aldrich. In preparation of the sample solution, the insulin powder was dissolved by dimethyl-sulfoxide (DMSO) with stirring at 200 rpm for 5 min. The solution was diluted by 100 mM phosphate buffer saline (PBS, pH 7.4) solution containing 100 mM NaCl. Final concentration of insulin was 100 μM. The volume fraction of DMSO and PBS was 1 : 19. This solution (500 μL) was poured into microtube (1.5 mL vol.).

The home-built experimental system for ultrasonic irradiation experiment is shown in **Fig. 1**. In this system, water bath was filled with degassed water for avoiding generation of cavitation bubbles there and then loss of the acoustic energy. Temperature of degassed water was kept at 37 °C. The ultrasonic transducer with fundamental frequency of 26 kHz was set in the bottom of the water bath. Then the microtube containing insulin sample solution was set above the transducer. We irradiated ultrasonic wave with five various amplitudes to the solution. A single ultrasonication sequence consisted of 1-min ultrasonic irradiation

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and 4-min incubation. This 5-min sequence was repeated for 30 min. The aggregation reaction was monitored by ThT fluorescence assay: 5 μL insulin sample solution was mixed with 50 μL ThT solution with concentration of 5 μM in a quartz crystal cell. 450-nm wavelength was used as the excitation light. Wavelength range from 440 to 500 nm was scanned, and the maximum value was recorded as the measurement.

The acoustic pressure of ultrasonic wave in the tube containing the buffer solution was measured with handmade PZT probe. Obtained data was analyzed with Fast Fourier Transform (FFT). The temperature change in the tube caused by ultrasonic irradiation was measured by radiation thermometer every 5 s.

3. Result and discussion

Time course of ThT fluorescence intensity is shown in **Fig. 2**. When the input power exceeds 120 W, aggregation reaction is immediately completed within 30 min, and the saturated ThT level appears to be independent of the input power. Below 120 W, the fibrillation reaction is not induced within 30 min. This indicates that the accelerated aggregation reaction of insulin induced by ultrasonic irradiation has a threshold power.

Temperature changes of the buffer solution in the tube caused by ultrasonic irradiation are shown in **Fig. 3**. Comparing between 120 W and 100 W, their temperature changes are similar, but aggregation reaction only occurred at 120 W. Thus, temperature is not principal for the acceleration of aggregation reaction induced by ultrasonic irradiation.

FFT spectra at 130 kHz (5th overtone) measured with the homemade PZT probe are shown in **Fig. 4**. Comparing between 120 W and 100 W, there is clear difference between their intensity. Harmonic components correlate with cavitation bubble dynamics. The higher acoustic intensity of harmonic component is, the more active cavitation bubbles are. So this indicates that when the conditions related with cavitation bubble exceed the threshold value, aggregation reaction is induced.

4. Conclusion

Not the temperature increase but the cavitation bubble is dominant for the aggregation reaction. This reaction is completed within 30 min at the optimized frequency. Thus, this study first confirms that cavitation bubble as the accelerator in the mechanism again. For insulin, this accelerated aggregation reaction occurs when the harmonics amplitude, which is related with cavitation bubble generation, exceeds a threshold value.

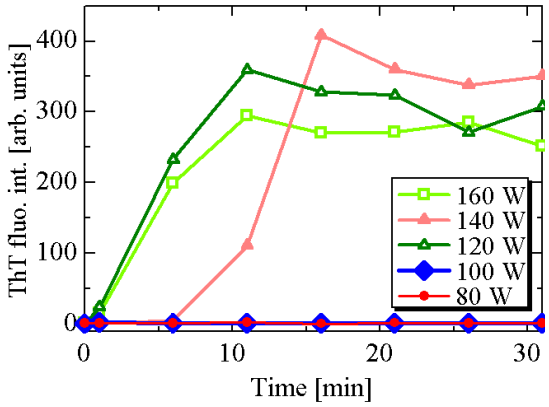

Fig. 2 Time courses of ThT fluorescence intensity of insulin sample caused by ultrasonic-wave irradiation with various powers.

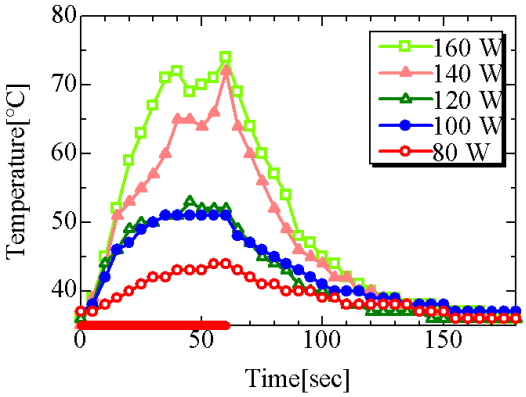

Fig. 3 Temperature changes of the buffer solution in the tube. The thick red line up to 60 s shows the irradiation time.

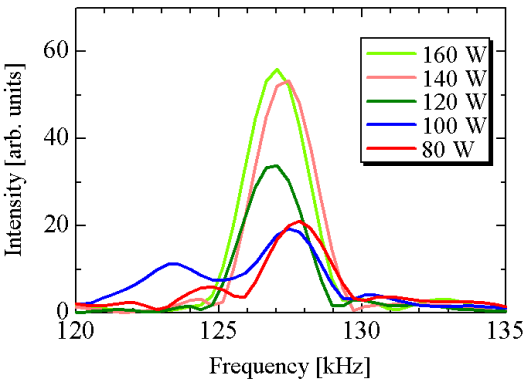

Fig. 4 FFT spectra at 130 kHz (fifth hamonics) at different ultrasonic powers.

References

1. R. J. Bateman *et al.*: N. Engl. J. Med. **367** (2012) 795.
2. M. Kihara *et al.*: J. Biol. Chem. **280** (2005) 12012.
3. K. Nakajima *et al.*: Sci. Rep. **6** (2016) 22015.