Improvement of detection ability for amyloid fibril seeds by interaction between ultrasonic cavitation and surfactants

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1. Introduction

Amyloidosis, as represented by Parkinson's, is caused by the formation of insoluble protein aggregates called amyloid fibrils in the body. Due to the nature of the disease, by the time clinical symptoms such as cognitive impairment appear, the formation of amyloid fibrils in the body has already completed and neuronal cells have already been exposed to irreversible damages. Hence, a radical cure is difficult. The process of amyloid fibril formation can be divided into two major steps. The first step is nucleation, in which soluble monomeric proteins form the seeds of amyloid fibril in vivo over a long period of time (several decades). The second step is fibril elongation, in which monomeric proteins bind one after another to the seed termini, and the reaction proceeds rapidly. In other words, nucleation is the rate-limiting step of amyloid formation. Therefore, to prevent the onset of amyloidosis, it is important to detect amyloid fibril seeds in vivo before the onset of clinical symptoms. However, the amount of amyloid fibril seeds formed before the onset is subtle, making detection difficult.

In 2020, it was reported that using α-synuclein, which is responsible for Parkinson's disease, a subtle amount of amyloid fibril seeds in patient specimens could be selectively amplified and detected by repeating incubation and shaking sequence, successfully discriminating patients and healthy individuals[1]. However, this method requires more than 300 hours per assay, making it challenging to adopt in early diagnosis, where a large number of specimens must be processed in a short time.

To solve this problem, we focused on the effects of ultrasound irradiation on amyloid fibril formation. It has been shown that ultrasonic irradiation with optimized conditions significantly accelerates amyloid nucleation compared to the shaking stimulus, and that the detection of fibril seeds is also highly sensitive[2,3]. The enhancement of amyloid fibril formation by ultrasound irradiation is due to the motion of cavitation bubbles generated in the solution. It is expected that the detection ability of amyloid fibril seeds can be further improved by controlling the characteristics of cavitation bubbles.

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In this study, we investigated the effects of combining ultrasonic irradiation and surfactants on the detection of α-syn amyloid fibril seeds. Previous studies have reported that ultrasound irradiation of a solution containing sodium dodecyl sulfate (SDS) causes the specific distribution of sonoluminescence generated by cavitation bubbles to spread uniformly[4]. The interaction between surfactants and cavitation is expected to affect the detection ability of amyloid fibril seeds. In this study, we investigate the detection ability of α-syn amyloid seeds under ultrasound irradiation using various surfactants, SDS, sodium decyl sulfate, sodium octyl sulfate (SOS). They are anionic surfactants with the number of hydrocarbon groups of 12, 10, and 8, respectively.

2. Experimental Method

In this study, α-syn monomers expressed in *Escherichia coli* were purified by liquid-phase chromatography. For the experiment, the α-syn monomers were diluted by sodium phosphate buffer with pH of 7.0 to be the monomer concentration of 0.1 mg/mL. The solution also includes 300 mM sodium chloride, and 5 μM thioflavin-T (ThT) dye. ThT was used for monitoring the time course of amyloid fibril formation because ThT molecule specifically binds to β-sheet structure of amyloid fibril and emits high intensity fluorescence. The preformed α-syn seeds were added to the monomer solution with a concentration of 3 ng/mL.

For the ultrasonic irradiation, we used the original sonoreactor equipment, HANABI system, as shown in **Fig. 1**[3]. A commercially available 96 well plate containing sample solution is sealed with a plastic film with 0.1 mm thick, and 30 piezoelectric transducers are individually placed on the film. A

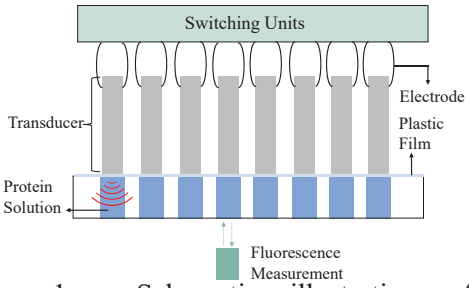

Fig. 1 Schematic illustration of the sonoreactor for amyloid fibril assay.

chirp burst voltage signal was applied to each transducer with an amplitude of 160 $V_{p\text{-}p}$ and frequency of 29.5-30.5 kHz. During the ultrasound experiment, the sample solution was repeatedly irradiated with ultrasound for 0.5 s and stopped for 30 s. The ThT-fluorescence intensity was measured every 5 min from the bottom surface of the each well.

3. Results and Discussion

We measured the time course of ThT fluorescence intensity of samples with and without seeds in the presence and absence of SOS, as shown in **Fig. 2**. With or without the addition of SOS, the amyloid fibril-formation is accelerated in the monomer solution by the addition of seeds. However, without SOS, the time-course curve with the seeds is not clearly distinguishable from that without the seeds because of the low seed concentration (Figs. 2(a) and (b)). While the addition of with SOS, were more clearly separates them(Figs. 2(c) and (d)). This indicates that SOS improves the seed-detection ability under ultrasound irradiation. In this paper, the lag time is defined as the time when the fluorescence intensity of ThT exceeds 100, and we use it for the quantitative evaluation of the detection ability. As shown in the **Fig. 3**, in the absence of SOS, the average lag times without and with seeds were 4.53±0.59 h and 3.5±1.69 h, respectively. Whereas in the presence of SOS, the average lag times were 5.64±0.59 h and 3.41±0.34 h, respectively. In addition, there is no overlap of lag time error bars with and without seeds. This indicates that the SOS improves the seed-detection ability.

The promotion of amyloid fibril formation in ultrasound irradiation is caused by two effects: fibril fragmentation and nucleation[2,5]. The former is caused by the shock wave during the crushing of

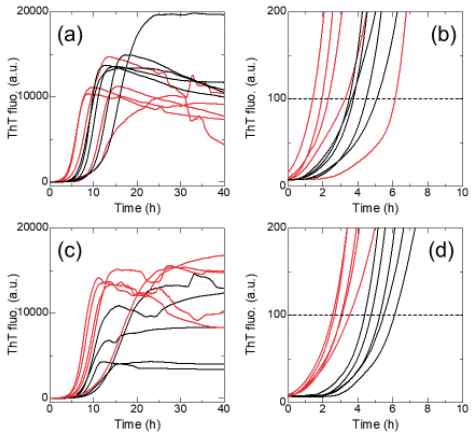

Fig. 2 Time-course curves of ThT fluorescence with SOS concentrations of (a)0 mM and (b) is its enlarged view, (c) 4 mM and (d) is its enlarged view, respectively. Black and red lines show ThT curves with and without seeds, respectively.

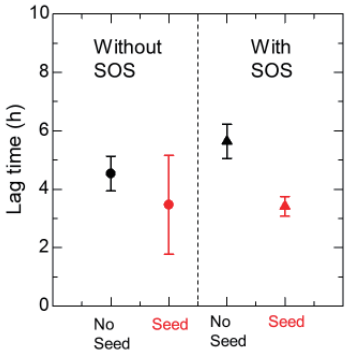

Fig. 3 Comparison of the lag time between α-syn solutions with and without seeds in presence and absence SOS.

ultrasonic cavitation, which can increase the seeds with active ends. Therefore, this reaction amplifies a small amount of fibril seeds and contributes to the seed detection ability. Whereas nucleation promotes the fibril seeds formation from monomers by adsorbing monomers on the surface of bubbles. Therefore, nucleation deteriorates the seed detection ability.

In the amyloid fibril formation induced by ultrasonic irradiation in the presence of surfactants, adsorption of the surfactants on the bubble surface prevents the adsorption of monomer molecules and presumably suppresses nucleation. Only the seeds-dependent fragmentation remained, which may have increased the seeds detection ability.

4. Conclusion

We investigated the effect of surfactants on amyloid fibril formation using α-syn, cause protein of Parkinson's disease. The experiments were performed using the originally developed multichannel sonoreactor. The results indicate that the detection ability of amyloid fibril seeds under ultrasound irradiation is improved by the addition of SOS.

Acknowledgments

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