Effect of surfactant on detection sensitivity of amyloid fibril seeds under ultrasonic irradiation

Kichitaro Nakajima[1,2†], Hajime Toda[1], Keiichi Yamaguchi[1,2], Kensuke Ikenaka[3], Hideki Mochizuki[3], Yuji Goto[1,2], and Hirotsugu Ogi[1] ([1]Grad. Sch. Eng., Osaka Univ.; [2]Global Center Med. Eng. Info., Osaka Univ.; [3]Grad. Sch. Med., Osaka Univ.)

1. Introduction

Amyloid fibrils are protein aggregates with needle-like morphology and relate to intractable diseases called amyloidosis. Each amyloidosis is caused by amyloid fibrils composed of a specific protein. For example, the cause proteins of Alzheimer's and Parkinson's disease (PD) are amyloid β peptides and α-synuclein (α-syn), respectively. Although these proteins usually exist as innocuous soluble monomers, the monomers form amyloid fibrils at an early stage of amyloidosis, eventually causing the disease by their toxicity. The previous study reported that amyloid fibril formation begins over ten years ago from the appearance of the clinical symptoms. Since the toxicity of amyloid fibrils leads to irreversible damage to biological tissues, detecting amyloid fibril seeds in vivo is crucial for preventing the onset of amyloidosis. However, the amount of amyloid fibril seeds formed at the early stage is subtle, making the detection of seeds challenging.

For the detection of amyloid seeds, several methodologies have been developed in the past two decades. Real-time quaking-induced conversion (RT-QuIC) is one of the promising methods for the detection of amyloid seeds [1]. In the RT-QuIC, the seeds are added to the solution which contains the monomers same as the constituent of the seeds. The mixed solution is shaken to rapidly amplify the seeds by binding the monomers to the termini of the seeds. Monitoring the time course of amplification of the seeds by means of fluorescence measurement using thioflavin-T (ThT), an amyloid-specific dye, allows us to distinguish the samples with seeds from ones without seeds. Indeed, previous studies reported success in distinguishing cerebrospinal fluid samples of PD patients from those of healthy controls based on RT-QuIC [2]. However, it has not been utilized yet as a diagnostic method in a clinical scene mainly because of two challenges, that is, long time for the detection over 300 hours and sensitive detection with high reproducibility.

To solve these challenges, we have focused on the accelerative effects of ultrasonication on amyloid fibril formation, have developed an optimized sonoreactor for amyloid-fibril assays, and have investigated the effects of ultrasonication on amyloid fibril formation depending on the seeds [3]. The series of studies revealed that ultrasonication can accelerate amyloid fibril formation greater than shaking agitation and that the detection sensitivity of amyloid seeds by ultrasonic irradiation is greater than that by shaking. These advantages are due to the effects of ultrasonic cavitation generated in a protein solution [4], suggesting that controlling the characteristics of cavitation bubbles can further develop the potential for ultrasonication-based amyloid seed detection.

In this study, we investigate the effects of surfactants on the detection of amyloid seeds under ultrasonic irradiation. Previous studies reported that adding sodium dodecyl sulfate (SDS), an anionic surfactant, to the liquid irradiated with ultrasonic wave modulates the spatial distribution of ultrasonic cavitation in a concentration-dependent manner because of the interaction between SDS molecules and cavitation bubbles [5]. Given that SDS molecules can interact with ultrasonic cavitation, it might affect the detection sensitivity of amyloid seeds under ultrasonic irradiation. Herein, we added SDS molecules with different concentrations to the solution of α-syn monomers and analyzed the detection sensitivity of α-syn amyloid seeds under ultrasonic irradiation.

2. Experimental Methods

α-syn monomers were expressed using *Escherichia coli* and purified by liquid-phase chromatography. The purified monomers were lyophilized and stocked at -20 °C. For the experiment, the α-syn monomers were diluted by sodium phosphate buffer with pH of 7.4 to be the monomer concentration of 0.1 mg/mL. The solution also contained sodium chloride and ThT dye with concentrations of 300 mM and 5 μM, respectively. The preformed a-syn seeds were added to the monomer solution with a concentration of 1 μg/mL.

For the seed detection experiment under ultrasonic irradiation, we used the optimized multichannel sonoreactor, which we have developed for amyloid fibril assays [3]. As shown in Fig. 1, the sonoreactor equips ultrasonic transducers composed of piezoelectric lead zirconate titanite (PZT) with the

E-mail: k.nakajima@prec.eng.osaka-u.ac.jp

resonant frequency of ~30 kHz, an optimum frequency for acceleration of amyloid fibril formation [5]. Alternating voltage with the amplitude of 100 Vp-p was applied to each PZT transducer with a duty ratio of ~1%. To monitor the time course of amyloid fibril formation, the ThT fluorescence was automatically measured every 5 min.

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 SDS, as shown in Fig. 2a and 2b, respectively. Regardless of the presence of SDS molecules, amyloid seeds (red lines) accelerated amyloid fibril formation compared with the monomer solution without seeds (black lines) due to the seeding effect. However, in the presence of SDS with the concentration of 0.1 mM, the ThT curves with and without seeds were more clearly separated compared with the results obtained without SDS, indicating that the addition of SDS improved the capacity of ultrasonication to detect the amyloid seeds.

Then, we quantitatively evaluated the effects of the addition of SDS on seed detection. As an indicator for the evaluation, the lag-time ratio, which is a ratio of the lag time of monomer samples divided by that of seed samples, was used. The lag time was defined as a time when the ThT value starts to increase. A larger lag-time ratio indicates a greater capacity of detection sensitivity of amyloid seeds. As shown in Fig. 2c, the lag-time ratio depended on the SDS concentration, demonstrating that the SDS molecules modulated the seed-detection capacity of ultrasonication. The SDS with concentrations of 0.1 and 0.3 mM highly improved the seed detection capacity of ultrasonication.

We previously reported that ultrasonic irradiation accelerates primary nucleation and fragmentation pathways in amyloid fibril formation. Although enhancement of fragmentation of amyloid fibrils by ultrasonic irradiation contributes to shorting the time for the seed detection, acceleration of primary nucleation deteriorates the detection sensitivity of amyloid seeds. Here, SDS might inhibit ultrasonic enhancement of primary nucleation, which occurs on the surface of cavitation through the hydrophobic interaction between protein monomers and bubble surface, by changing the bubble surface from hydrophobic to hydrophilic nature. This resulted in the improvement of seed detection capacity of ultrasonic irradiation. To further leverage the SDS molecules to amyloid seeds detection under ultrasonic irradiation, it is necessary to consider the surfactant effects on protein molecules because it can directly modulate amyloid fibril formation through the interaction with proteins [6].

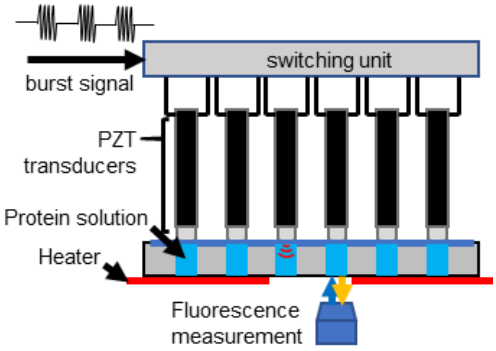

Fig. 1. Schematic illustration of the sonoreactor for amyloid fibril assays.

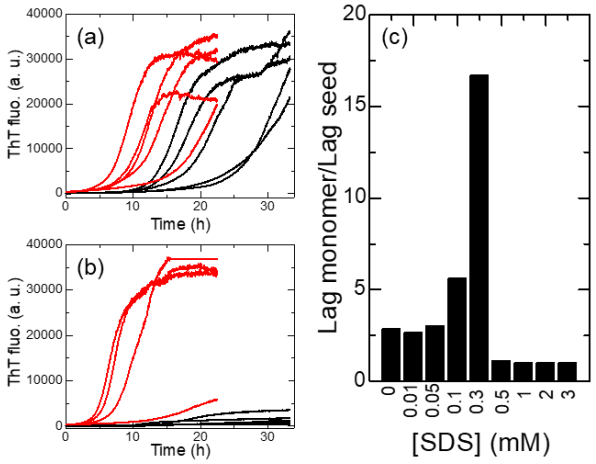

Fig. 2. Time-course curves of ThT fluorescence with SDS concentrations of (a) 0 and (b) 0.1 mM, respectively. The red and black curves show the ThT curves of samples with and without seeds. (c) Ratio of the lag times between samples with and without seeds. Larger values indicate greater seed detection sensitivities.

4. Conclusion

We investigated the effects of SDS on detection sensitivity of amyloid seeds using α-syn, cause protein of Parkinson's disease. The experiments were performed using the originally developed multichannel sonoreactor. The experimental results indicated that the addition of SDS affected the detection sensitivity of amyloid seeds under ultrasonic irradiation, being attributed to the interaction between the SDS molecules and cavitation and/or protein molecules.

References

1. R. Atarashi et al.: *Prion* **5** (2011) 150-153.
2. M. Shahnawaz et al.: *Nature* **578** (2020) 273-277.
3. K. Nakajima et al.: *Ultrason. Sonochem.* **73** (2021) 105508.
4. K. Nakajima et al.: *Sci. Rep.* **6** (2016) 22015.
5. J. Lee et al.: *J. Phys. Chem. B* **114** (2010) 2572-
6. M. Sawada et al., *Langmuir* **36**, (2020) 4671-4681.