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Specific effect of ultrasonic irradiation on amyloid-fibril formation reaction

アミロイド線維形成反応に対する超音波照射の特異的効果

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1. Introduction

Amyloid fibrils are proteinaceous aggregates, which possess an ordered structure with needle-like morphology. The amyloid-fibril formation in vivo causes serious diseases, called amyloidosis. Although there are various kinds of amyloidosis with different causative proteins, the underlying onset mechanism shows commonality: Functional soluble protein monomers transform their state into insoluble amyloid fibrils through an aggregation reaction. The amyloid fibrils damage biological tissues by their toxicity. Because it is difficult to completely cure the tissues once damaged, an early-stage diagnosis of amyloidosis is essential for mitigating the diseases.

Recently, aggregation-acceleration methods based on mechanical agitations have attracted significant attention for the early-stage diagnosis of amyloidoses[1,2], because they dramatically shorten the time for investigating the fibril-formation propensity of target protein molecules derived from biological fluids. As the mechanical agitation, ultrasonication and shaking are widely adopted to accelerate the fibril formation reactions. Although they may result in different effects on the fibril-formation reaction, the difference remains unclarified.

In this study, we systematically investigate the aggregation reaction of β_2-microglobulin (β2m), which is a causative protein of dialysis-related amyloidosis, under ultrasonication and shaking. Because the fibril-formation reaction under the ultrasonic field is highly sensitive to acoustic conditions, it is difficult to investigate the aggregation reaction in a reproducible way. Then, we use an originally developed sonoreactor, which is optimized for the amyloid-fibril assay[3], and compare ultrasonic effects on the fibril-formation reaction with the shaking effects. In the experiments, we perform the fibril-formation assay using acidic β2m monomer solution to investigate the effects of ultrasonication and shaking on the spontaneous amyloidogenic aggregation reactions. In addition to the spontaneous fibril formation, we investigate the agitation effects on a seeding reaction, which occurs in protein solutions including preformed fibrils as a template. The aggregation-reaction kinetics is measured by the thioflavin-T (ThT) fluorescence assay. The structure and morphology of the resultant aggregates are investigated by circular dichroism (CD) spectroscopy and atomic force microscopy (AFM) observation, respectively.

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2. Experimental Methods

We previously developed the optimized sonoreactor for the amyloid-fibril assay[3], as schematically shown in Fig. 1, combined with a fluorescence plate reader. The sample solutions including β2m monomers are placed in a commercially available 96-well plate with a sample volume of 198 μL. The solution is sealed by a plastic film with a thickness of 0.1 mm. The piezoelectric lead-zirconate-titanate (PZT) transducer is placed on each sample solution one-by-one. The resonant frequency of the PZT transducer is ~30 kHz, which is the optimum frequency for accelerating nucleation reaction of the fibrils[4]. Acoustic couplant was adopted to increase the propagation efficiency of the ultrasound. The resonance spectrum of each transducer was measured by a microphone beneath the 96-well plate. During the ultrasonic experiment, each solution was irradiated with the ultrasound for 0.3 s with an interval of 30 s, and the ThT fluorescence intensity of each sample was measured every 10 min.

In the shaking assay, we used a commercially available fluorescence plate reader equipping a

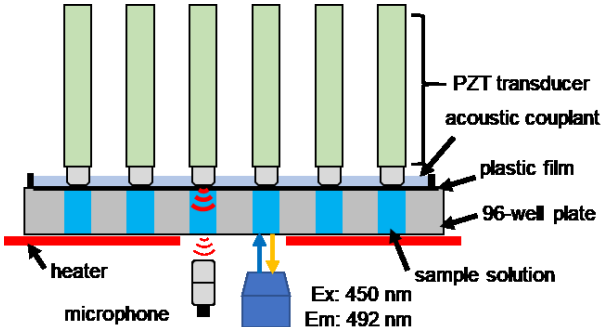

Fig.1. Schematic illustration of on optimized sonoreactor for the amyloid-fibril assay[3].

shaking function. The shaking agitation with the revolution of 850 rpm was performed for 20 second every 10 min.

In this study, β2m monomers expressed in *Escherichia Coli* BL21 were purified and used for the experiments. For the fibril-formation experiment, we prepared sample solutions including β2m monomer with various concentrations (0.01-1.0 mg/mL), 20 mM HCl, 100 mM NaCl, and 5 μM ThT.

3. Results and Discussion

We first investigated the morphology of the aggregates formed by shaking and ultrasonication, as shown in Figs. 2(a) and 2(b). While both of them exhibit fibril-like morphology, the fibrils formed by ultrasonication have a short and dispersed morphology due to the fragmentation effect of ultrasonication[5].

Next, we prepared the sample solutions with different β2m monomer and NaCl concentrations, performed the fibril-formation experiment using shaking and ultrasonication, and analyzed the fibril-formation reaction in terms of the reaction time to form the fibrils. Figures 3(a) and 3(b) shows the reaction-time distribution under shaking and ultrasonication, respectively. In the region of no aggregates in Fig. 3, the monomer concentration is lower than the solubility, meaning that the aggregation reaction never occurs. At high monomer and NaCl concentrations, the morphology of resultant aggregates shows the amorphous aggregates but not amyloid fibrils, as indicated by the amorphous-aggregates region in Fig. 3. Within the region where amyloid fibrils form, ultrasonication exhibits a higher aggregation-acceleration effect than shaking, especially for the monomer solution with a low aggregation propensity. This fact indicates the high efficiency of the ultrasonic aggregation acceleration which is attributed to the local condensation of protein monomers by ultrasonic cavitation[6].

4. Conclusion

In this study, we systematically investigated the effects of shaking and ultrasonication on the amyloid-fibril formation reaction. The AFM observation of aggregates demonstrated the difference in the resultant morphology, indicating the difference in the effect of the agitations on the amyloid fibrils. Furthermore, the systematic reaction-time analysis revealed a higher efficiency of the ultrasonic aggregation acceleration than that of shaking. This difference in the effect on the aggregation reaction is attributed to the difference in the aggregation acceleration mechanism of ultrasonication and shaking.

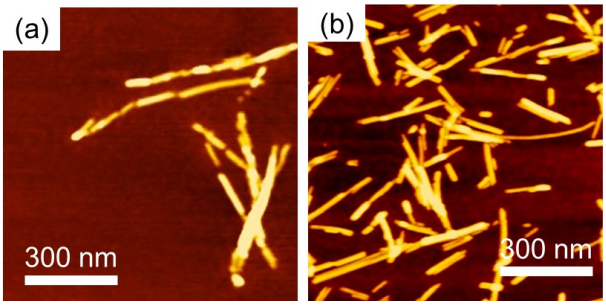

Fig.2. AFM images of amyloid fibrils formed by (a)shaking and (b)ultrasonication.

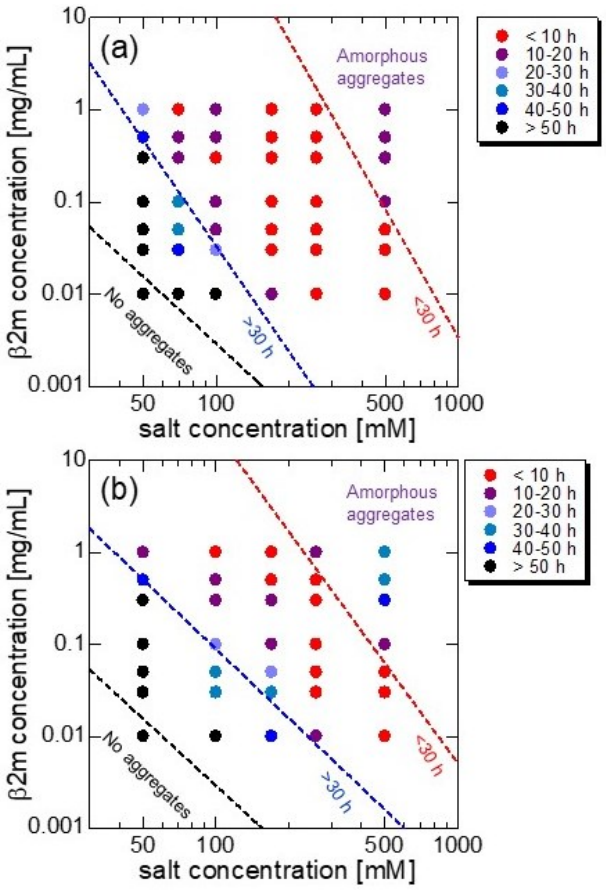

Fig.3. The fibril-formation time in different solution conditions under (a)shaking and (b)ultrasonication.

References

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