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Study on aggregation reactions of amyloid β peptides induced by ultrasonic irradiation and stirring agitation

超音波と撹拌により誘導されるアミロイドβ蛋白質の凝集反応の研究

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1. Introduction

Because amyloid β (Aβ) peptides form neurotoxic aggregates in vivo, the aggregation reaction is deeply related to onset and development of Alzheimer's disease (AD). The aggregation reaction takes very long term, preventing us from clarifying the mechanism. The aggregation mechanism, therefore, remains unclear, and there is no effective treatment for AD.

It has been reported that aggregation reactions of some peptides, including Aβ peptide, are drastically accelerated by ultrasonic irradiation to their solutions (1, 2). In this reaction, the aggregation reaction is completed within a few hours by ultrasonic wave, which would otherwise take much longer time. The morphology of aggregates produced by ultrasonic-wave irradiation is principally composed of amyloid fibrils, which is similar to those observed in the AD-patient brain.

In this study, we, thus, researched the aggregation mechanism of Aβ peptide with ultrasonic wave. The ultrasonic irradiation experiments were performed with home-built experimental system. Stirring experiments, which is conventionally used as aggregation-acceleration method, was also performed for comparison. Their aggregation behaviors were compared with each other using the fluorescence measurement of thioflavin-T (ThT), which specifically binds to amyloid fibrils and emits strong fluorescence. Their morphologies were also compared an atomic force microscopy (AFM).

Ultrasonic irradiation to human body is often applied in medical diagnosis, such as monitoring of an unborn child. Aβ peptide exists as monomer state in blood. If the aggregates are formed by ultrasonic irradiation to the body, they might cause AD. Understanding of ultrasonically induced aggregation phenomenon of Aβ peptides, thus, leads not only to clarification of the onset mechanism of AD, but also to discussion about safety usage of ultrasonic wave in cases of treatment and diagnosis.

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2. Methods

Lyophilized powder $A\beta_{1\text{-}40}$ peptide was purchased from Peptide Institute (4307-v). In preparation of the sample solution, the Aβ powder was dissolved by dimethyl-sulfoxide (DMSO). The solution was then diluted by 100 mM phosphate buffer saline (PBS, pH 7.4) solution containing 100 mM NaCl. Final concentration of Aβ peptide was 10 μM. The volume fraction of DMSO and PBS was 1 : 4.

For ultrasonic irradiation experiment, we developed a homebuilt experimental system. In this system, water bath was filled with degassed water for avoiding loss of acoustic energy by scattering caused by the cavitation bubbles. Temperature of the degassed water was kept at 18 °C by a homebuilt temperature control system. The ultrasonic transducer with fundamental frequency of 26 kHz was set to the bottom of the water bath. Sample tubes containing the 10-μM Aβ solutions were set above the transducer and irradiated with ultrasonic wave through the degassed water.

In the ultrasonic experiment, 1-min ultrasonic irradiation and 9-min incubation are alternately repeated. This 10-min sequence was applied, and the ThT fluorescence level was measured every 30 minutes by obtaining a 5-μl Aβ solution and mixing it with ThT solution in the quartz crystal cell, which was set to a spectrophotofluorometer to measure its fluorescence intensity: The sample was exited with wavelength of 450 nm, and emitted light was scanned from 440 nm to 500 nm. Because ThT shows the emission peak near 485 nm, the maximum intensity in the scanned range was recorded as the ThT fluorescence level. As control experiment, an incubation experiment was performed at 37 °C. The 800-rpm stirring procedure was also investigated as the other mechanical agitation to induce the aggregation reaction.

The morphologies of the Aβ aggregates caused by ultrasonic irradiation and stirring agitation were observed by AFM: A 5 μl solution was dropped onto freshly cleaved mica plate, dried for 15 min,

rinsed by ultrapure water (50 μl), and dried for 15 min to make the substances in the solution attached on the mica plate. The tapping-mode measurement was adopted with a silicon cantilever with the stiffness of 40 N/m, showing the resonance frequency near 300 kHz.

3. Results

We performed aggregation experiments with ultrasonic irradiation, stirring, and incubation procedure. Time course of the ThT level caused by each procedure is shown in Fig. 1. The aggregation reaction was not observed for 10 h in the incubated sample (open circles) despite higher temperature, indicating a high energy barrier of the aggregation reaction. With ultrasonic wave (open diamonds), formation of the aggregates occurs around 5 h. Its ThT curve is composed of three phases: The nucleation phase, where the ThT level appears to be unchanged, the fibril-growth phase with drastic increase in the ThT level, and the saturated phase. This is a typical aggregation behavior for the amyloid-fibril formation. The bundle of fibril is observed in its AFM image, shown in Fig. 2 (a). On the other hand, the aggregation reaction is induced with no lag by the stirring procedure (open squares). In the AFM image, amorphous aggregates are mainly observed as shown in Fig. 2 (b). From these results, the amyloid fibrils and amorphous aggregates are principally induced by ultrasonic irradiation and stirring procedure, respectively.

We also researched whether amyloid fibril formed by ultrasonic irradiation and amorphous aggregate caused by stirring procedure change their conformation by stirring procedure and ultrasonic irradiation, respectively. Solid squares in Fig. 1 show the evolution of the ThT level when the stirring agitation was first applied for 3 h and then ultrasonic irradiation was applied. There was no noticeable change in the ThT level with the high-power ultrasonic irradiation. This indicates that the amorphous aggregates cannot transform into fibrils. In the opposite case, where ultrasonic wave was applied first for 3 h, and the stirring procedure followed, significant increase occurred in the ThT level with the stirring effect.

4. Discussion

Formation of amyloid fibril is accompanied with nucleation reaction, which possesses high energy barrier (3). Ultrasonic irradiation induces formation of amyloid fibrils by decrease of an apparent energy barrier for nucleation. Formed amyloid fibril has stable and regular structure called cross-β-sheet structure. Thus, it is not induced conformation change by stirring procedure which possesses lower energy by ultrasonic wave.

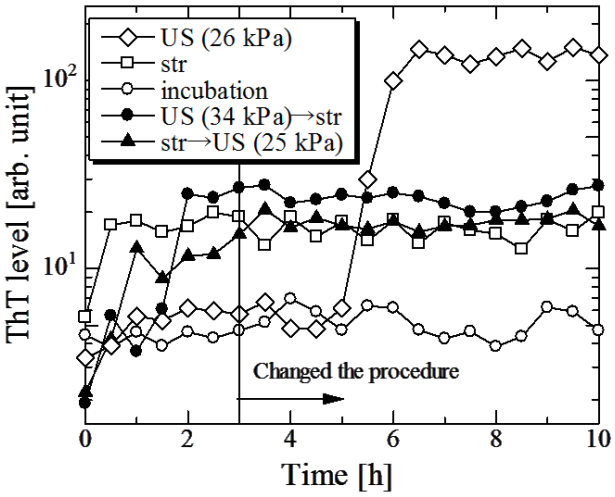

Fig. 1 Time courses of ThT level caused by ultrasonic irradiation (US), stirring (str), and incubation procedures.

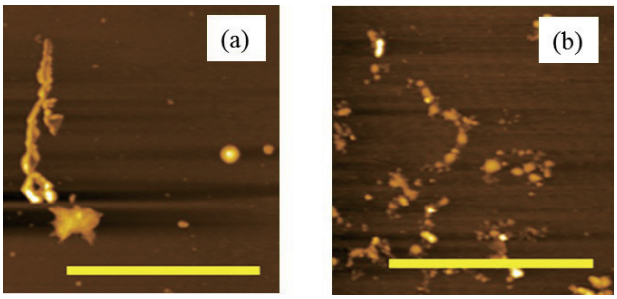

Fig. 2 AFM images of aggregates formed with (a) ultrasonic irradiation and (b) stirring procedure. Scale bars indicate 5 μm.

Amorphous aggregates are immediately formed by the stirring procedure, indicating lower energy barrier for formation of amorphous aggregates than it of amyloid fibrils. The amorphous aggregates, however, possesses stable conformation because of they are not destroyed by ultrasonic irradiation. The reason of which an amorphous aggregate is not formed by ultrasonic irradiation might be that weak bind to form amorphous aggregates is decomposed by ultrasonic wave, and rigid aggregates such as nucleus of amyloid fibril is preferentially formed under ultrasonic field.

References

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