2P5-9

Proceedings of Symposium on Ultrasonic Electronics, Vol. 34 (2013) pp. 317-318
20-22 November, 2013

Effect of glycerol on ultrasonically induced aggregation phenomenon of amyloid β peptides

アミロイドβペプチドの超音波誘起異常凝集現象におけるグリセロールの効果

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1. Introduction

Today, dementia derived from Alzheimer's disease (AD) is a critical issue in the aging society. One of the pathological characteristics of AD is a senile plaque (SP) on the cerebral cortex, which consists of deposits of amyloid-β (Aβ) aggregates [1]: The pathogenic mechanism of AD is deeply related with the aggregation mechanism of the peptides. The Aβ peptides consist of ~40 amino acid residues with molecular weights of about 4 kDa. Aβs are released from amyloid protein precursor by two proteases, β- and γ-secretases [2]. They form aggregates, including oligomers and well-oriented amyloid fibrils. The amyloid fibrils have β-sheet structures, where the β strands are aligned with regularity. Although it is believed that neurotoxicity in AD is caused by SP containing aggregates of Aβ, the aggregation mechanism remains unclear, decelerating development of effective approaches to AD.

The fact that it generally takes decades to develop AD indicates that the nucleation for aggregation of Aβ possesses a high energy barrier, preventing us from clarifying the aggregation mechanism. Therefore, it is significantly valuable on AD's study to develop a methodology to accelerate the aggregation of Aβ.

Previous studies showed that ultrasonication to various peptides (α-synuclein [3] and β_2-microglobulin [4]) induces amyloid fibrils. We recently found that the aggregation of Aβ peptides is accelerated with ultrasonication to a large extent as well [5]. However, the mechanism of ultrasonically induced aggregation phenomenon is still unclear. We indicated that cavitation bubbles generated by ultrasonication in the solution are strongly related to the nucleation process of aggregates [5]. Thus, we study relationship between ultrasonically induced aggregation phenomenon and viscosity of solution by altering concentration of glycerol to change properties of cavitation bubbles. The viscosity significantly affects the bubble dynamics, and also we expect the apprent degree of supersaturation of the peptide because glycerol occupies the solvent.

In this study, we used $A\beta_{1\text{-}40}$ peptides. The ultrasonic transducer with fundamental frequency of 26 kHz was used to perform ultrasonication experiments. The thioflavin-T (ThT) assay was adopted for monitoring the formation of the β-sheet structures. ThT selectively binds to β-sheet structures and emits high intensity fluorescence. The ThT assay is thus effective for monitoring the formation of amyloid fibrils. The morphology of amyloid fibrils was observed by AFM. The water used to propagate the ultrasound was degassed for avoiding bubble generation there.

2. Experimental Procedure

The lyophilized Aβ was dissolved in dimethyl sulfoxide (DMSO) with stirring at 200 rpm for 10 min and then, diluted by phosphate buffer saline (PBS) solution, including 0.1M NaCl and glycerol (0, 2, or 5 %) to obtain the final concentration of the peptide to be 10 μM (DMSO : buffer solution containing glycerol = 1 : 4). This solution was poured in the microtubes (500 μl) and we performed ultrasonication for them. A single ultrasonication sequence consisted of 1 min ultrasonication and 9 min incubation. This 10 min sequence was repeated, and ThT assay was performed every 30 min. As control experiments, the stirring and incubation experiments were also preformed. In the stirring experiments, Aβ solution was stirred at 800 rpm by a magnetic stirrer.

The ultrasonic transducer was located in a water tank, which was filled with degassed water. The temperature in the water tank was kept under 15 °C. Several microtubes (1.5 ml vol.) containing 500 μl sample solution were located above the transducer at different locations so as to perform the ultrasonication measurement with various acoustic pressures simultaneously. The acoustic pressure was measured by a handmade needle-type PZT probe, which was calibrated by a 1.0 mm diameter needle-type hydrophone.

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Nucleation and fibril formation were evaluated using the ThT assay. ThT was dissolved into 50 mM glycine-NaOH buffer (pH 8.5) to obtain the final concentration of 5 μM. The obtained solution was wrapped in aluminum foil and stocked at 4 °C before use. From the examining solutions, which were subjected to ultrasonication, stirring, and incubation, aliquots of 5 μl were taken every 30 min and mixed with 50 μl of ThT solution in quartz cell. At fluorescence measurement, we used excitation light with 450 nm, and scanned the detected wavelength from 450 to 500 nm, measuring the fluorescence intensity. The maximum fluorescence intensity in the scanned wavelength range was then recorded as fluorescence intensity. The measurement was performed every 30 min.

The morphologies of the Aβ aggregates obtained by ultrasonication, stirring and incubation were observed using an atomic-force microscopy (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. The scan frequency was 0.5 kHz.

3. Results and Discussion

Figure 1 shows changes of the ThT fluorescence with progress of the ultrasonication for the three glycerol concentrations. We previously displayed that the acoustic pressure of second harmonics wave has showed a high correlation to ultrasonically induced aggregation phenomenon [5]. The acoustic pressures of second harmonics wave were 39.1 kPa (0% glycerol), 34.3 kPa (2% glycerol) and 37.6 kPa (5% glycerol). Despite of the nearly identical acoustic pressure of the second harmonics wave, the ThT fluorescence was clearly dependent on the glycerol concentration. On the other hand, it showed the same tendency for all concentrations of glycerol in the case of the stirring experiment as shown in **Figure 2**.

The aggregation by the stirring procedure is simply caused by enhancement of association between Aβ molecules. However, the temperature increase and local condensation of the peptides caused by the bubbles are expected in ultrasonically procedures. Thus, obtained results indicate that ultrasonically induced aggregation phenomenon is significantly influenced by the glycerol concentration, and it will be possible to find a suitable concentration of the glycerol to accelerate the aggregation of Aβ with a very high efficiency.

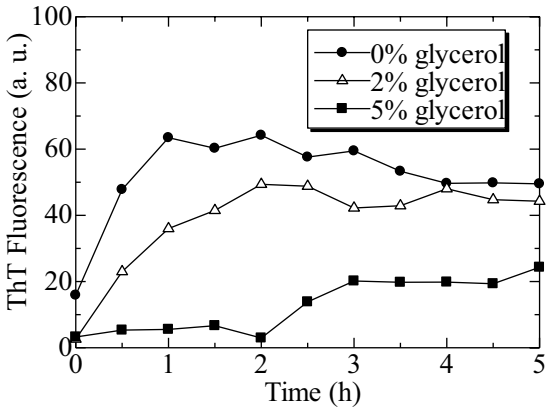

Fig.1 Change in the ThT fluorescence intensity in Aβ solution with ultrasonication (26 kHz).

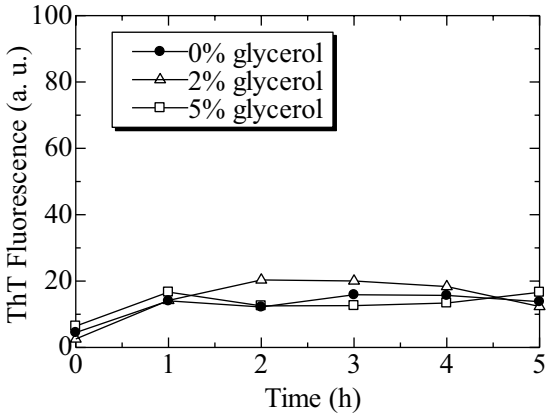

Fig.2 Change in the ThT fluorescence intensity in Aβ solution caused by the 800 rpm stirring.

4. Conclusion

By investigating the aggregation of Aβ when the concentration of glycerol is altered, we revealed that the viscosity of the reaction solution affects the ultrasonically induced aggregation behavior of $A\beta_{1\text{-}40}$ peptides. From this result, finding a suitable concentration of the glycerol to accelerate more significantly the aggregation of Aβ contributes to study of AD.

References

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