Study on dynamic characteristics of acceleration effect of Amyloid β peptide aggregation by shear stress field

せん断応力場によるアミロイドβペプチドの凝集加速効果の動特性研究

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1. Introduction

Alzheimer's disease (AD), which is the most prevalent cause of dementia, affects over 50 million worldwide [1]. One of the primary pathological characteristics of AD is the extracellular aggregation and deposition of Amyloid β (Aβ) peptides. Aβ peptides are proteins which consist of 39-43 amino acid residues, and they aggregate from monomer to amyloid fibrils. There are a lot of studies focusing on Aβ peptides' aggregation behavior. However, the detailed characteristics of aggregation have not been fully elucidated. One of the principal reasons is the fact that the aggregation reaction takes a long time. The development of an aggregation acceleration method is, therefore, an important issue.

In Newtonian fluid, shear stress is proportional to the velocity gradient. In the brain of AD patients, Aβ peptide is thought to first form aggregation nuclei on nerve cells, on which the Aβ monomers that have entered the bloodstream are adsorbed, resulting in forming amyloid fibrils [2]. In other words, a shear-stress field due to blood flow is always generated in the aggregated nucleus. Here, we propose a new acceleration method by enhancing the shear stress using a piezoelectric resonator and develop a microscopy system for monitoring the aggregation reaction under the shear stress application using total internal reflection fluorescence microscope (TIRFM).

Figure 1 illustrates the experimental system we developed. We used a Z-cut lithium niobate ($LiNbO_3$: LN) resonator to cause the shear-stress field near the surface. Because of its piezoelectricity, it can be oscillated contactlessly when an oscillating electric field is applied by an antenna. When a shear resonance is generated, the shear-stress field is produced near the oscillator surface via the viscosity of the contact fluid. TIRFM is a type of fluorescence microscopy that uses evanescent light as an excitation light. It enables imaging phenomena that occurs only near the surface and highly sensitive detection of fluorescent molecules with little background light. Fluorescence emitted from an aggregate is detected by utilizing a specific fluorescent substance that absorbs excitation light and emits fluorescence with a longer wavelength. By introducing the specific fluorescent reagent into the target molecule, the dynamic behavior of the aggregation reaction can be observed via fluorescence with the presence of the shear-stress field. In this study, we used thioflavin T (ThT), which fluoresces by binding specifically to the β-sheet structure protein aggregations. Since amyloid fibrils have many β-sheet structures, they are used as fluorescent labels for amyloid fibrils. Using this system we monitored Aβ peptides' aggregation in real time (**Figure 1**).

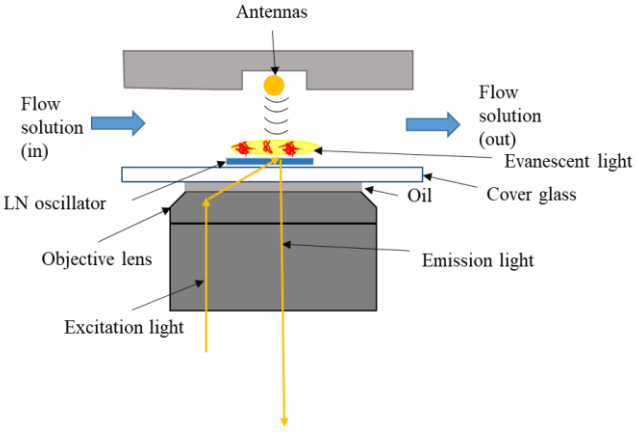

Figure 1 Schematic illustration of the experimental system.

2. Experiment Procedure

In this study, we used $Aβ_{1-42}$ as aggregated nuclei and $Aβ_{1-40}$ as flow solution. A 10 μM $Aβ_{1-42}$

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amyloid-fibril solution was sonicated to make fragments of the fibril seeds (aggregated nuclei). Then, the $A\beta_{1-42}$ seed solution including 30 μM ThT was injected on the LN oscillator, which was washed by the piranha solution (H_2O_2:H_2SO_4 = 3:7) and cleaned by UV for 15 min in advance, and incubated for 10 h at 4 ℃ to immobilize the seeds on the LN surface. A 10 μM $A\beta_{1-40}$ monomer solution including 30 μM ThT was then flowed during exciting ultrasonic vibration, and the aggregation process on the LN oscillator was monitored with TIRFM.

3. Result and Discussion

Figures 2 and 3 show the fluorescent images taken by TIRFM without and with the shear stress application, respectively. **Figure 4** shows the change of the occupancy rate of the amyloid fibrils obtained during formation of the amyloid fibrils. Without the shear stress, the amyloid fibril was not generated until ~240 min, and very short fibrils appeared at ~600 min. However, an explosive increase in the amyloid fibrils was observed from 70 min by applying the shear stress, and the fibrils continued to grow. Up to now, such fibril development has not been reported, which strongly suggests the effectiveness of the shear-stress field. Furthermore, the length of the fibrils made with shear stress application seem to be longer than the fibrils made without shear stress application. That is, the shear-stress field appears to make fibrils longer.

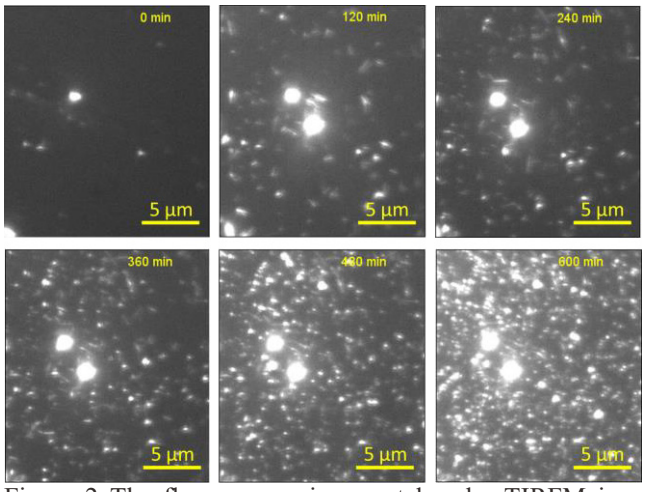

Figure 2 The fluorescence images taken by TIRFM in forming Aβ amyloid fibril without the shear stress application.

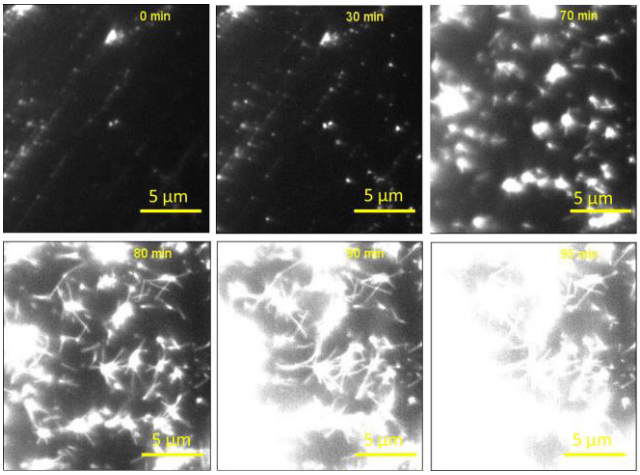

Figure 3 The fluorescence images taken by TIRFM in forming Aβ amyloid fibril with the shear stress application.

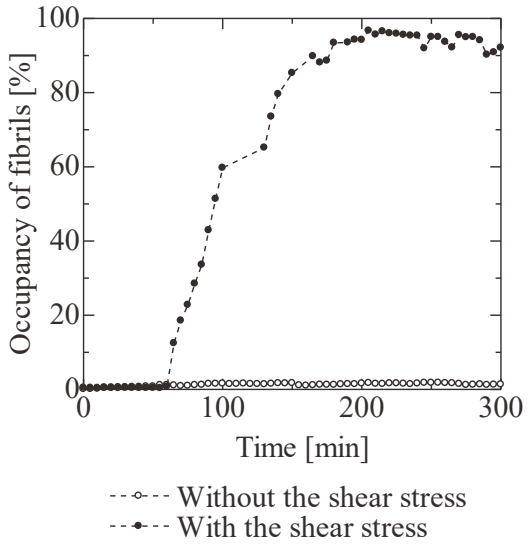

Figure 4 The change of the occupancy rate of the amyloid fibrils obtained from TIRFM images without (open circles) and with (solid circles) the shear stress application.

4. Conclusion

We succeeded in monitoring the dramatic acceleration of Aβ peptides' aggregation under the shear-stress field by TIRFM. Furthermore, the shear-stress field also affects the fiber elongation behavior. We will make experiments for other proteins which form amyloid fibrils.

5. References

1. C. Patterson, "World Alzheimer report 2018" (Alzheimer's Disease International, London, 2018)
2. H.Ogi, Y.Fukunishi, T.Yanagida, H.Yagi, Y.Goto, M.Fukushima, K.Uesugi and M.Hirao, Anal. Chem., 83, 4982 - 4988 (2011)