Direct observation of aggregation reaction of α-synuclein under shear stress using total internal reflection fluorescence microscopy with lithium-niobate resonator

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1. Introduction

Most proteins normally fold into specific, stable tertiary structures to perform their physiological functions. However, when proteins misfold for some reason, they can form aggregates. Amyloidogenic proteins form such aggregates as oligomers and amyloid fibrils, which are responsible for the development of various diseases called amyloidosis [1]. Parkinson's disease (PD) is a typical amyloidosis, and it has been known that aggregates of α-synuclein, a protein composed of 140 amino acids, cause the disease [2-4]. However, the mechanism of aggregation reaction of α-synuclein is still not fully understood. One reason is that the aggregation reaction takes a very long time. Furthermore, the aggregation reaction is considered to be a surface reaction in vivo, in which soluble monomers are deposited on the aggregation seeds immobilized on the membrane tissue inside and outside the cell. Therefore, there has been a need to develop an instrument that promotes the aggregation reaction on a surface and monitors its progress in real time. In this study, we propose a methodology for accelerating the aggregation reaction of α-synuclein on a lithium-niobite (LN) surface using ultrasonically induced shear-stress agitation.

Ultrasonication is recognized as a powerful tool for dramatically accelerating aggregation reactions of various proteins [5, 6], relying on the cavitation bubble dynamics [7]. In this study, we also use ultrasound, but focus on the shear stress. The blood flow in vivo causes the shear stress near the tissue surface, which should promote the aggregation reaction. We use an LN oscillator and cause the shear resonant vibration to generate the shear stress near the surface, on which the aggregation reaction proceeds. We integrate the LN resonator with an optical microscopic system and excite its shear vibration contactlessly using antennas, making the real-time observation of the aggregation reaction made possible because of the transparency of LN. In this study, we investigate the effect of the shear-stress agitation on the fibril-elongation reaction of α-synuclein. We develop a multipoint total-internal-reflection fluorescence-microscopy (TIRFM) system, which can acquire time-lapse images at multiple regions on the LN surface, providing more information than previous studies that monitored fibril growth behavior at a single location [8, 9]. We believe that this method will contribute to clarification the aggregation process of the protein.

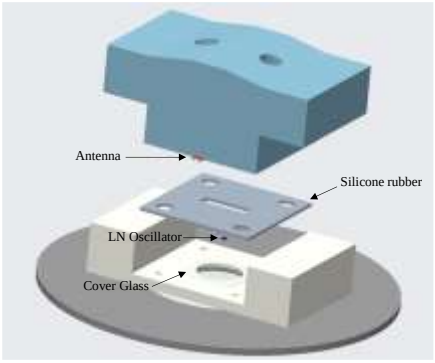

Fig. 1 Appearance of originally developed TIRFM flow-cell system with an LN oscillator.

2. Experimental Methods

The flow cell originally developed here is shown in Fig. 1. We used a Z-curt LN resonator with a thickness of about 40 μm, which was immobilized on a 120-μm thick cover glass. The flow channel was made of 1 mm thick silicone rubber, and the oscillator was set on the cover glass. We prepared the seed solution by irradiating a 0.5 mg/ml monomer solution with ultrasound for a sufficient time. We then immobilized the seeds on the LN oscillator by dripping the seed solution onto the substrate surface with a pipette and incubated at room temperature for 3 h. After washing with a buffer solution, the top of the flow cell was fixed with bolts. The monomer

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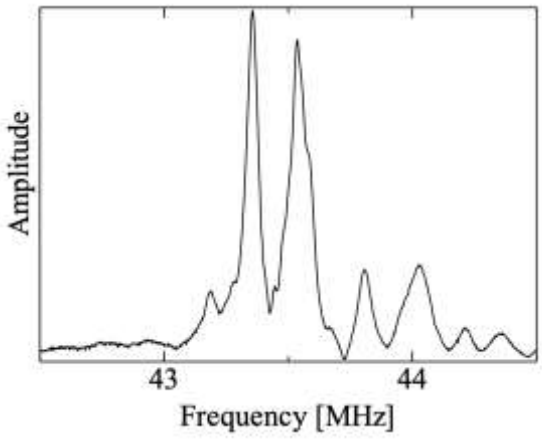

Fig. 2 Resonant spectrum of the LN oscillator during the solution flow measured by the non-contacting antennas.

solution (concentration of 0.3 mg/ml) with 50-μM thioflavin T (ThT), which is a dye widely used to visualize the amyloid fibrils, was then flowed with a flow rate of 200 μl/min at 37°C. The shear resonant vibration of the LN oscillator was excited by the antenna shown in Fig. 1. The elongation behavior of the amyloid fibrils was thus monitored under the shear stress induced the by LN oscillator.

3. Results and Discussion

First, the resonance frequency of the LN oscillator was confirmed by applying a tone-burst voltage to the generation antenna, sweeping its frequency, and detecting the vibrational amplitude through the detection antenna. Figure 2 shows the resonant spectrum, showing resonant peaks near 43.5 MHz, being consistent with the estimation for the through-thickness shear-wave resonant frequency of the LN oscillator using the reported material constants [10]. Therefore, the measurement system developed here is capable of applying the shear-stress agitation near the LN surface during the fibril elongation reaction with monomer solution flow. Assuming the Newtonian fluid for the solution, the shear-stress field is expected to exist up to ~100 nm from the LN surface from the viscosity, density, and the resonant frequency, and considering the fibril diameter of ~20 nm, this shear-stress region will be effective as the stimulus for promotion of the aggregation reaction.

Figure 3 shows snapshots of the fibril elongation reaction on the LN oscillator. There was no noticeable change in the TIRFM image for ~60 min after injection of the monomer solution, but about 100 min later, many fibers were observed to elongate from originally immobilized seeds. Some fibrils also appeared form the newly created seeds.

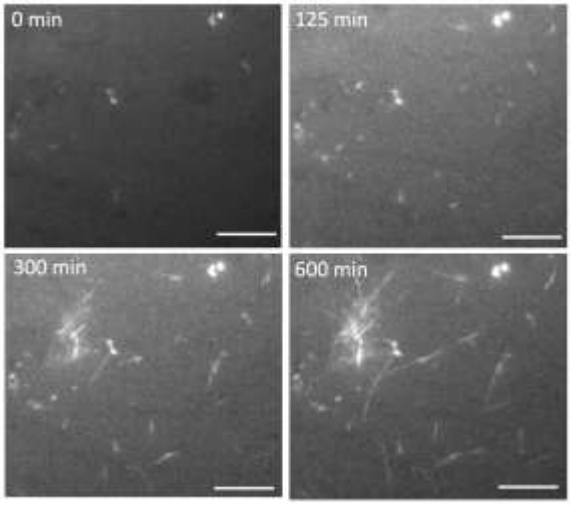

Fig. 3 Snapshots of TIRFM images on the LN oscillator during fibril elongation reaction of α-synuclein. Scale bars indicate 5 μm.

The elongation rate was then increased, and the fibril elongation reaction ceased when the fibril length reached about 5 μm. The cessation of the elongation reaction is not due to the monomer depletion, because we kept supplying monomers by a continuous flow of the monomer solution. The fibril-elongation behavior appears to be different, depending on the shear-stress value, which will be shown in more detail in the symposium.

4. Conclusion

In this study, we have originally developed a multipoint TIRFM system with the wireless-electrodeless LN resonator, where the aggregation reaction of proteins on surface can be monitored under the shear-stress stimulus. This system is expected to contribute to the elucidation of protein aggregation reactions and also to the development of drugs that inhibit the aggregation reaction or dissolve the aggregates.

References

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