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Study on viscoelastic properties of amyloid-fibril network during formation and disaggregation by wireless quartz crystal microbalance

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1. Introduction

Amyloidosis is a fatal disease caused by accumulation of aberrant protein aggregates, called amyloid fibrils, which seriously affect human life activities. There are various kinds of proteins that form amyloid fibrils in vivo, causing the corresponding amyloidoses. For example, aggregates of α-synuclein (α-syn) are involved in Parkinson's disease, multiple system atrophy, and so on. α-syn presumably contributes to the regulation of cellular functions, but in these diseases, it forms aggregates, showing neurotoxicity. However, the mechanism by which α-syn forms aggregates remains unclarified. Previous studies indicated that the aggregation process of α-syn is affected by the cell membrane, suggesting the importance of investigating the aggregation process at a solid-liquid interface[1].

In this study, we investigate the aggregation process of α-syn at a solid-liquid interface using a quartz crystal microbalance (QCM) method. Our laboratory originally developed the wireless-electrodeless QCM (WE-QCM) method, possessing the advantage of high sensitivity for protein detection[2,3]. The QCM method can measure the aggregation process in a label-free manner. By leveraging these advantages of the WE-QCM method, we performed surface seeding experiments for amyloid fibrils and evaluated the viscoelastic properties during the protein-layer-formation process. We also tried to evaluate the viscoelasticity change by injecting proteolytic enzymes, which possess the ability to digest amyloid fibrils.

2. Wireless-electrodeless QCM biosensor

Experiments were performed using a multichannel WE-QCM biosensor, which we originally developed. **Figure 1** shows a schematic illustration of the experimental setup with the WE-QCM experiment. Conventional QCM devices have metal electrodes on both sides of a quartz crystal resonator. However, they deteriorate the detection sensitivity for the change in a surface mass because they increase the dynamic mass of the quartz crystal resonator. The WE-QCM overcomes this problem. This system enables the quartz crystal resonator to oscillate without electrodes by exciting it with an electromagnetic field emitted from antennas. This drastically decreases the mass of the quartz crystal, thereby increasing the sensitivity and allowing the detection of target molecules with high sensitivity.

In this study, we used quartz crystal resonators with fundamental resonance frequencies near 64.5 MHz with dimensions of 2.5 mm × 1.7 mm × 26 μm. We developed the four-channel WE-QCM system that allows monitoring of different aggregation reactions in the same flow channel[4]. We set four quartz crystal resonators in the microchannel, and the time course of their resonant frequencies was monitored by pairs of antennas, as shown in Fig. 1.

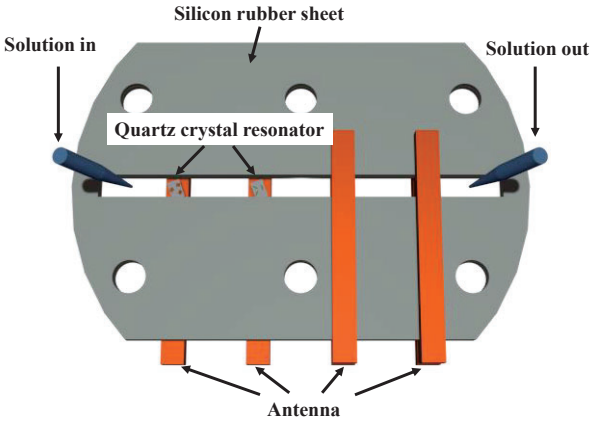

Fig. 1 Schematic illustration of the WE-QCM biosensor system.

3. Experiments

Lyophilized α-syn monomer was dissolved by Tris-HCl buffer (50 mM, pH 7.4) supplemented with 50 mM NaCl. The α-syn concentration was adjusted to be 0.5 mg/ml by measuring the optical absorbance at 280 nm. This solution was used to prepare amyloid fibrils, which were sonicated with 47.5 kHz ultrasound prior to the experiment and used as a seed solution. The α-syn monomer solution was prepared with a concentration of 0.3 mg/ml in Tris-HCl (50 mM, pH 7.4) supplemented with 250 mM NaCl.

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Before the QCM experiment, the quartz crystal resonator was washed with a piranha solution which is composed of hydrogen peroxide and sulfuric acid (H_2O_2:H_2SO_4=3:7) for 5 min and sufficiently rinsed with ultrapure water. Then, the resonators were placed between silicon rubber sheets, and then, diluted amyloid protein seeds or bovine serum albumin (BSA) was immobilized on each resonator surface by incubating overnight. Here, BSA was used as a negative control to confirm the ability of amyloid seeds. After the surface of the quartz crystal resonator was adequately washed with buffer solution, the resonators were placed in a laboratory-made sensor holder, and then, buffer solution was circulated using a piezoelectric micropump with a flow rate of 18 μl/s. We first measured the resonance frequencies of the resonators and after they stabilized, we injected the target solution and monitored the phase changes while the solution circulated. The time course of the resonance frequency was calculated by assuming the linear relationship between the resonance frequency and the phase near the resonant frequency, and the viscoelastic properties were evaluated. We also performed atomic force microscope (AFM) observation on the quartz crystal surfaces before and after the flow measurements.

4. Results and discussion

Figure 2 shows the resonant frequency changes during the α-syn monomer solution circulated. The red, green, and blue curves show the resonant frequency change of the quartz crystal resonators on which 300, 30, and 3 μg/ml of α-syn seed solution were used for the seed immobilization, respectively, and the orange one shows the resonant frequency change of the quartz crystal resonator blocked with 1 mg/ml of BSA. The experimental results show that the higher the concentration of immobilized α-syn seed, the greater decrease in resonant frequency change. This indicates that the number of fibrils which composes the network through the seeding reaction is dependent on the initial surface density of seeds, resulting in a greater mass change. AFM images of the quartz crystal surfaces after the monomer solution flow showed differences in the number of fibrils network, consistent with the QCM results.

With fibril network formed on the quartz crystal resonator, we injected an inhibitor[5] of fibril formation and proteolytic enzymes, and measured the resonant frequency change while the solution circulated. The resonance frequency increased when each of them was injected. AFM observations performed after the QCM experiment showed that fibrils were dissolved by the addition of proteolytic enzymes, whereas many fibrils remained when inhibitors were added. This suggests that the increase in resonance frequency when proteolytic enzymes are added is due to the dissociation of the fibril network, causing a decrease in the mass, while the increase in the resonance frequency by the inhibitors is due to a change in the viscoelastic properties of the fibril network, indicating an increase in the fibril stiffness.

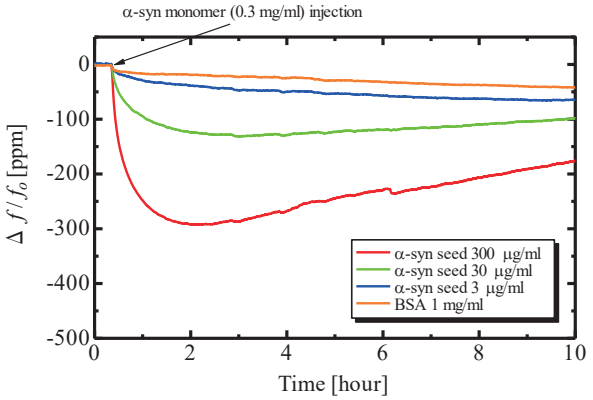

Fig. 2 Resonant frequency change after injection of α-syn monomer (0.3 mg/ml) monitored by the multichannel QCM biosensor.

5. Conclusion

We investigated seeding reactions of amyloidogenic proteins using the originally developed WE-QCM system at a solid-liquid interface. The seeding experiments at the interface showed the formation of various fibril networks depending on the seed concentration. After the formation of the fibril networks, we injected inhibitors and proteolytic enzymes into the fibril network and analyzed the characteristic viscoelastic changes. This viscoelastic property is unique to amyloid fibrils and may lead to the elucidation of the fibril formation process of amyloid.

Acknowledgment

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