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Monitoring of viscoelasticity and structural change during aggregation reactions of β–2 microglobulin with wireless quartz-crystal-microbalance biosensor

無線水晶振動子マイクロバランスによる β2m の凝集過程における粘弾性と構造変化の観察

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1. Introduction

The dialysis-related amyloidosis is one of systemic amyloidosis, and is a disease that causes carpal tunnel syndrome due to accumulation of amyloid fibrils throughout the body. It is caused by the deposition of amyloid fibrils of β_2-microglobulin (β2m). [1] It is a component of type I major histocompatibility complex, and a constant amount of β2m is released into the blood due to cell destruction. In a healthy person, it is filtered by the glomerulus of the kidney, reabsorbed, and broken down by the renal tubules. On the other hand, in a person with the renal dysfunction, the blood level of β2m increases, and β2m is deposited on bones and joints. By this mechanism, dialysis amyloidosis develops. However, the transformation mechanism of β2m monomers into a fibril structure remains unclear, which could be a key for the pathogenic mechanism of the dialysis amyloidosis. Therefore, we study the mechanism of the aggregation reaction of β2m from the fibril seeds from the viscoelasticity change of the formed aggregate.

The β2m is a small globular protein with a molecular weight of 11800 composed of 99 amino acid residues and is a single-chain polypeptide containing no sugar chain. On the other hand, amyloid fibrils have a needle-like structure with a diameter of 10 to 15 nm and a length of 1 to 2 μm. Amyloid fibrils are formed by a multimolecular polymerization reaction consisting of nucleation and elongation reactions. It is known that in the condition that only monomeric proteins are present, nucleation hardly occurs, however in the condition that amyloid fibril fragments already exist, the reaction proceeds rapidly. Here, in order to reveal the mechanism of the elongation reaction in the fibril─formation, the reaction between the fibril fragment and the monomer protein was monitored using the wireless quartz crystal microbalance biosensor.

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2. Wireless quartz-crystal-microbalance method

We prepared an AT-cut quartz resonator with an Au thin film on its surface. It vibrates at the resonance frequency when the electric field is applied from the antenna. When a target substance attaches to the QCM surface, the resonance frequency changes depending on its mass. Besides, the resonating amplitude decreases because of the viscoelasticity of the formed protein layer. We used QCM to monitor mass change and viscoelasticity change through changes in the phase and amplitude at the driving frequency. The resonator was a 1.8 mm×1.6 mm×26 μm AT-cut quartz crystal with 2 nm chromium and 8 nm gold film on one side, and it has a fundamental shear mode resonance frequency of 64 MHz. We used a wireless QCM without the electrodes in order to avoid the effects of the pressure difference between the liquid and the air and to make the crystal oscillator thinner. [2] It is possible to measure accurately the mass and viscoelasticity because the sensitivity is improved. Vibration excitation and detection were accomplished with the non-contacting antennas, so the other side of the resonator remained uncoated.

3. Experiment

First, we cleaned the crystal oscillator by the piranha solution, then washed it by ultrapure water and absolute ethanol. We secondly injected 10 mM 10-carboxy-decanthiol in absolute ethanol and incubated the crystal oscillator at 4 ℃ overnight to form the self-assembled monolayer (SAM). Next, we injected a mixture of 100 mM N-hydroxysulfosuccinimide sodium salt (NHS) and 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, hydrochloride (EDC) in ultrapure water after washing by absolute ethanol and ultrapure water in order to activate the SAM terminal. Then, we injected the β2m seed solution which were

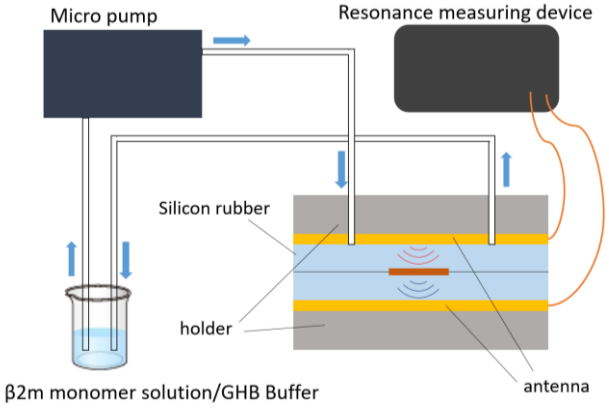

Fig. 1 Schematic illustration of the homebuilt experimental system.

prepared by breaking ultrasonically the β2m fibrils. The β2m seeds were then immobilized over 2 h. Finally, we washed the resonator by the 0.1M Glycine-HCl (GHB) buffer and injected 5 mg/mL bovine serum albumin (BSA) in GHB buffer to block the sites unbound to seeds. We held the crystal oscillator between silicon rubbers and installed QCM in a homemade sensor holder. Figure 1 shows the experimental setup. Then we flowed GHB buffer and circulated using a micropump to get a stable baseline. The flow rate was about 400 μL/min. Then, we flowed the prepared β2m monomer solution and monitored the phase and amplitude changes.

3. Result and Discussion

Figures 2 (a) and (b) show changes in the resonance frequency and peak amplitude, respectively. The red curves show their changes when the β2m monomer solution was injected on the resonator, on which the β2m seeds were immobilized, and the blue ones shows their changes on the sensor chip, where only BSAs were immobilized. A significant frequency decrease was observed within 15 minutes after injecting the monomer on the β2m -seed immobilized resonator (Fig. 2(a)), but we did not observe frequency decrease on the BSA-immobilized resonator. The amplitude change was also more significant for the injection of the monomer solution on the seeds (Fig. 2(b)). We calculated the viscoelasticity and observed significant mass and viscoelasticity changes after injecting the monomer on the BSA immobilized resonator. There is thus a specific interaction between the seed and the monomer. It is considered that this rapid change will be explained by the viscoelasticity change as well as the mass change. [3] The significant frequency decrease causes a large increase in viscoelasticity. There is a specific reaction between the β2m seeds and monomers.

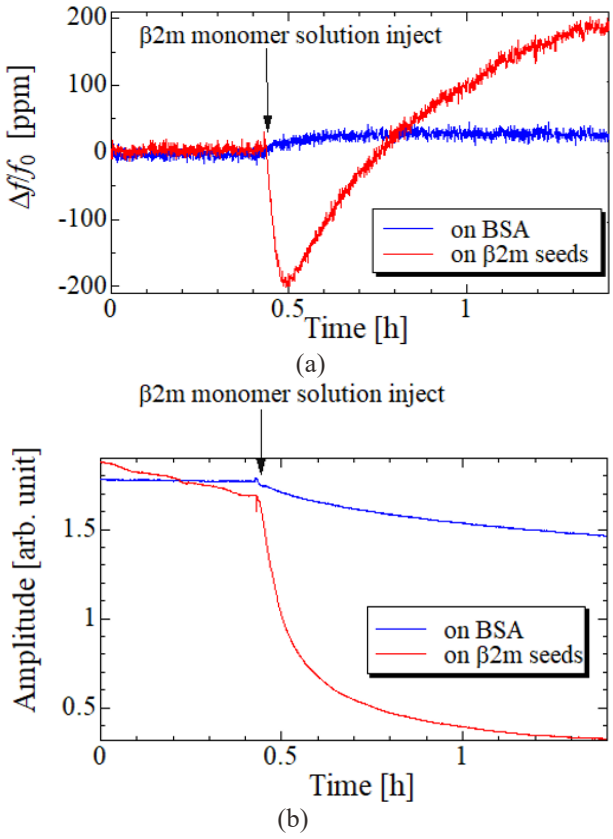

Fig. 2 Changes in (a) the resonant frequency and (b) peak amplitude by injecting the β2m monomer solution on the β2m -seed immobilized (red) and BSA immobilized (blue) resonators.

4. Conclusion

We observed the effect of viscoelasticity between the β2m seed and the monomer. After the experiment, we find that the interaction occurs only between the β2m seed and the monomer.

References

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