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Seed-Dependent Aggregation Behavior of Amyloidosis Peptides Studied by Wireless Quartz Crystal Microbalance Biosensors

凝集核に依存したアミロイドーシス誘起ペプチドの凝集過程のQCMモニタリング

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1. Introduction

Amyloidosis is a kind of condition, where amyloid proteins are deposited in organs, such as Alzheimer disease (AD) and dialysis amyloidosis (DA). Recent studies show that Aβ peptides and β2-microglobulin are causative peptides of AD and DA, respectively [1]. Concentrations of these peptides are controlled to be constant by enzymes, antibodies and metabolic organs inside the body. However, organ failure or some factors make concentrations of these peptides higher, and when they become oversaturated, these peptides begin to aggregate, which is believed to cause toxicity to organs. Although it is widely recognized that these aggregates of peptides are principal causes of AD and DA, the mechanism of aggregation remains unclear. Therefore, measuring the aggregation behaviors of these peptides plays an important role in the establishment of diagnosis and management of the amyloidosis.

In this study, we use the multichannel wireless quartz-crystal microbalance (QCM) biosensor to systematically study aggregation behaviors of these peptides. A QCM biosensor can measure recognition behavior among biochemical molecules by changes of the resonance frequency of the quartz-crystal resonator. When the receptor modified quartz-crystal resonator captures the receptor-specific molecules, the effective mass of the resonator increases. Then, the resonance frequency of the resonator decreases. Hence, we can monitor the binding reaction quantitatively in real-time. Furthermore, an ordinary aggregation experiment was made by stirring or shaking bulk solutions containing the peptides. In this method, however, the interaction between aggregates promotes and dominates further aggregation. In vivo, the first step in the aggregation process is depositing of amyloid nuclei on the surface of nerve cell. Then, monomers of the peptides aggregate on them [2]. In order to duplicate this condition, we immobilized amyloid nuclei on the surface of the quartz-crystal resonator, and flowed monomers to make interaction between monomer and the nuclei.

Only this method enables us to evaluate the aggregation property depending on the amyloid nuclei. However, a conventional low-sensitive QCM biosensor cannot pick up the aggregation of Aβ peptide or β2-microglobulin because of their low molar weight. To monitor the aggregation process, a higher-sensitive QCM is necessary. It can be achieved by making QCM thinner, wireless, electrodeless because the sensitivity is inversely proportional to the square of the thickness. Then, we can monitor the aggregation of these peptides using higher-sensitive QCM biosensor [3].

We monitored the aggregation behavior of Aβ peptide and β2-microglobulin depending on various amyloid nuclei by the QCM biosensor over 50 h. We form the different morphology of the nuclei by means of constant stirring or ultrasonication. We observed the morphologies of these nuclei and aggregates after the aggregation experiment by an atomic-force microscope (AFM).

2. Experimental procedure

Aβ1-42 was dissolved in a dimethyl sulfoxide (DMSO) solution and diluted by phosphate buffer saline (PBS, pH7.4) solution, acetate buffer saline (ABS, pH4.6) solution, or glycine-HCl buffer saline (GHB, pH2.2) solution to the concentration of 50 μg/ml. β2-microglobulin was dissolved in ultra pure water and diluted by HCl solution (pH2.5) to the concentration of 300 μg/ml. Each solution contains 100 mM NaCl. The various nuclei were formed in these peptide solutions with three methods of forming (stirring, ultrasonication, and ultrasonication fragment) and different pH conditions.

We used several AT-cut quartz-crystal plates whose fundamental resonance frequencies were about 43-60 MHz for the multichannel QCM biosensor. 18 nm Au films were deposited on both surfaces of each plate for the gold-alkanthiol binding reaction. In the following, we show how to immobilize the nuclei on the plates. First, the plates were cleaned in the piranha solution (98% H_2SO_4: 33% H_2O_2 = 3:7), and then rinsed with ultrapure

water. Next, they were immersed in a 10 mM 10-carboxy-1-pentanethiol solution for 12 h at 4 °C and activated by 100 mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide solution for an hour at 20 °C. Finally, they were immersed in the solution containing various amyloid nuclei for 4 h at 4 °C. These plates were set in the sensor cell, and it was incorporated in the flow-injection system (**Fig. 1**). A micropump ran the monomer solution at a flow rate of 500 μl/min. Monomer solution was circulated for a long-time (~50 h) aggregation experiment. The temperature of sensor cell was kept at 37 °C.
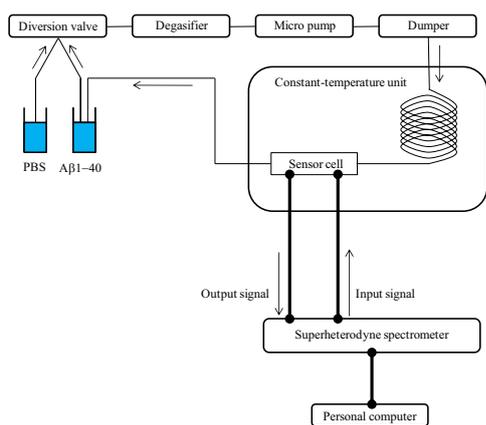

Fig. 1 Flow-injection system

3. Results and Discussions

Figs. 2 and **3** show examples of the change in the resonance frequency during the monomer flow. We succeeded at monitoring the difference of aggregation behavior depending on the nuclei. In the experiments of Aβ1-40 monomer flow on Aβ1-42 nuclei which are formed under the condition of pH 7.4, the amount of the deposited Aβ1-40 monomer is larger on the ultrasonicated nuclei than on the stirred one. **Figs. 4** and **5** show AFM images of each aggregate. Aβ1-40 forms different aggregates depending on the nuclei. **Fig. 4** indicates that the morphology of the nuclei is the dominant factor of the aggregation pathway. β₂-microglobulin did not form fibrils but amorphous-like aggregates.
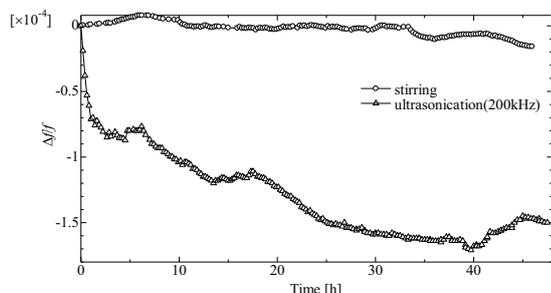

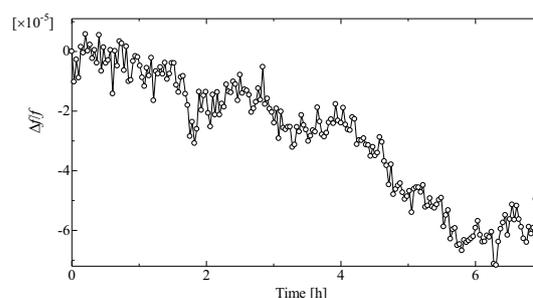Fig. 2 Changes in resonance frequencies during the aggregation of Aβ1-40 on the Aβ1-42 nuclei.

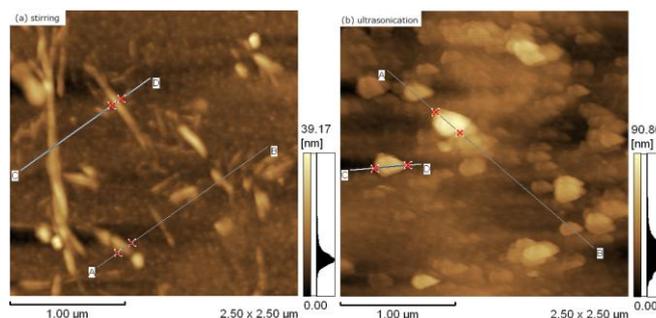Fig. 3 Changes in resonance frequency during the aggregation of β₂-microglobulin.

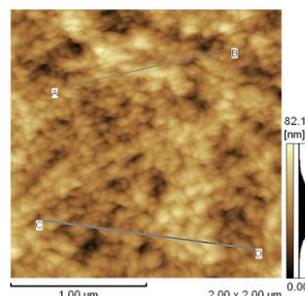Fig. 4 AFM images of morphology of Aβ1−40 aggregating on Aβ1−42 nuclei.

Fig. 5 AFM images of morphology of β₂-microglobulin aggregating on the nuclei.

4. Conclusion

We succeeded in monitoring the aggregation behavior of Aβ peptide and β₂-microglobulin. The aggregation property varies greatly according to the nuclei. This experiment is promising approach to identify what kind of nuclei contributes to highly toxic aggregate.

References
1. G. T. Westermark and P. Westermark: *Amyloid*, **7** (2000) 19.
2. A. Morinaga, K. Hasegawa, R. Nomura, T. Ookoshi, D. Ozawa, Y. Goto, M. Yamada, H. Naiki, *Biochimica et Biophysica Acta*, **1804** (2010) 986.
3. H. Ogi, K. Motohisa, T. Matsumoto, K. Hatanaka, M. Hirao, *Analytical Chemistry*, **78** (2006) 6903.