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Systematic Research on the Dependence of the Aggregation Behavior of Aβ Peptides on the Amyloid Nuclei Using Multichannel Electrodeless QCM

多チャンネル無電極 QCM を用いた凝集核形態に依存する Aβ ペプチド凝集能に関する研究

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1. Introduction

Alzheimer disease (AD) is a kind of dementia that causes the decline of cognitive faculty and change in the personal character. Recent intensive studies [1-3] confirm that Aβ peptide is the dominant pathogeny of AD. The concentration of Aβ peptide is normally controlled by specific enzymes and antibodies in the cortical area of the brain. However, several factors make the concentration of the peptide higher, and when it exceeds a critical value, agglutinates of Aβ peptides are formed, resulting in neurotoxicity and cell cytotoxicity [4]. Therefore, understanding of the aggregation mechanism of Aβ peptide is an important issue for the development of drugs for AD. Several previous studies reported relationships of amyloid nuclei and Aβ aggregation [5, 6]. But in those studies, marker proteins were used, which should have affected the aggregation behavior of the peptide because of the relatively small molecular size. Thus, label-free and quantitative monitoring of the aggregation process of Aβ peptides has not been achieved because of the measurement difficulty.

In this study, we develop the multichannel quartz-crystal microbalance (QCM) biosensor for studying aggregation behavior of Aβ peptides. A QCM biosensor has shown pronounced ability for studying recognition behavior among biochemical molecules through changes in the resonance frequency of the quartz plate. The surface-modified quartz plate adsorbs target molecules, resulting in the increase of the effective mass of the resonator and then in the decrease of the mechanical resonance frequency, which can be monitored during the binding reactions in real time without any labels. The QCM is a mass-sensitive biosensor, and its sensitivity deteriorates for Aβ peptides, because Aβ has a low molar weight (~4 kDa). Thus, it has been required to improve the QCM sensitivity. Most convincing approach is making the quartz-plate thickness diminished, because the QCM sensitivity is inversely proportional to the square of the thickness. We have then proposed a wireless-electrodeless technique for this and achieved advanced QCM systems with much higher fundamental frequencies up to 170 MHz [7-9].

Here, we study the dependence of the aggregation behavior of Aβ peptides on the morphology of the amyloid nuclei using the multichannel wireless QCM biosensor with 48-55 MHz fundamental resonance frequencies. Aβ peptides take several configurations depending on its aggregation pathway, and it is important to identify the nuclei on which the peptide aggregates with a higher rate. In order to form the different configuration of the nuclei, we use the stirring method, which has been known as an effective tool to control the Aβ morphology [11]. We used the stirring times of 0, 6, 12, 24, and 90 h.

2. Experimental Procedure

We used five AT-cut quartz plates with thicknesses of 30, 31, 32, 34, and 35 μm for the multichannel QCM biosensor, whose fundamental resonance frequencies were near 55, 53, 51, 48, and 47 MHz, respectively. For the gold-alkanethiol binding reaction, we deposited 18-nm Au films after 2-nm Cr films on both surfaces of the quartz plates. The crystals were cleaned in the piranha solution ($98\%H_2SO_4$:$33\%H_2O_2$=3:7), and after rinsing with ultrapure water, they were immersed in a 10 mM 10-carboxy-1-pentanethiol solution for 24 h. Their surfaces were then activated using a 100 mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) solution for 1 h. Then, the crystal plates were immersed in several solutions containing the different 1-42-Aβ-peptide ($Aβ_{1–42}$) seeds, which were produced as follows: Lyophilized $Aβ_{1-42}$ was dissolved in a dimethyl sulfoxide (DMSO) solution and diluted by PBS to obtain the final concentration of 50μg/ml. The solution was

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then stirred for 0, 6, 12, 24, or 90h, which were used for the immobilization on the five different sensor chips. The sensor crystals were set in the handmade sensor cell, and the sensor cell was incorporated in the homebuilt flow-injection system. The micropump provided a constant flow of the buffer solution (PBS; pH7.4) at a flow rate of 500 μl/min. The solution flowed sensor cell through degasifier and 2 m Teflon tube that made the solution temperature stable at 37ºC.

The rf bursts were applied to the generation antenna to generate the shear-horizontal vibrations in all the quartz plates in the noncontacting manner. After the excitation, the reverberating signals were received by the receiving antenna, which entered into the superheterodyne spectrometer to measure the phases and amplitudes of individual sensor chips. As the analyte to be injected, we used a 50 μg/ml $A\beta_{1\text{-}42}$/PBS solution.

3. Results

Fig. 1 shows an example of observed resonance-frequency changes during the aggregation of the Aβ monomer. We succeed in monitoring the long-time aggregation behavior of the Aβ peptide, and its dependence on the difference of the peptide seeds. After the injection, the resonance frequency monotonically decreased, and its decrement was larger for the aggregation on well grown nuclei. After the flow injection measurement, we investigated the structure of aggregates using atomic force microscopy, and found that only the aggregate on the nuclei stirred for 90 h formed the amyloid fibril.

4. Discussion

According to the Sauerbrey equation [10], the rate of the change in the resonance frequency equals the rate of the absorbed mass of the protein to the oscillator mass. The expression is shown as follows.

$$\frac{\Delta f}{f} = -\frac{\Delta m}{M} \qquad (1)$$

Here , f , Δf, M, and Δm denote the fundamental resonance frequency, its change, mass of the oscillator, and mass of the adsorbed protein on the oscillator, respectively. According to this equation, the amount of the Aβ peptide that adsorbed on the oscillator is calculated. For example, when stirring time is 90 h, aggregation amount for 20 h is calculated as 38 ng. This leads to the aggregation rate that 388 $A\beta_{1\text{–}42}$ monomers will agglutinate in the brain on the area of 1 nm^2 every year.

We have observed two important aggregation behaviors. First, the amount of aggregated peptide increases as the stirring time grows. The aggregated

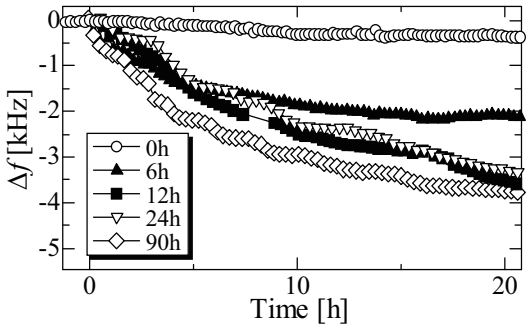

Fig. 1 Frequency responses during the aggregation of the $A\beta_{1\text{-}42}$ peptide. The horizontal axis shows the time measured after $A\beta_{1\text{-}42}$ arrived at the sensor cell.

amounts for 20 h are 38, 216, 367, 342, and 388 ng for 0, 6, 12, 24, and 90 stirring times, respectively. Secondly, we observe the stepwise aggregation behavior. The step period seems to be larger as the stirring time increases, and also it seems to be larger as the aggregation time increase. Thus, measuring the aggregation behavior of Aβ peptides quantitatively in real time can provide us with important information about the inherent biochemical property of Aβ peptides, and such behavior should be known in the development of drugs for AD.

5. Conclusion

We developed a high sensitive multichannel electrodeless QCM, and succeed in monitoring the aggregate behavior of $A\beta_{1\text{–}42}$ without labeling. The aggregation rate significantly depends on the configuration of the nuclei, and the step-wise aggregation behavior appeared. The wireless-electrodeless multichannel QCM is thus contribute to the study of the aggregation mechanism of Aβ peptides.

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