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Dynamic characterization of amyloid-fibril formation of Amyloid β peptide using total-internal-reflection fluorescence microscopy coupled with quartz-crystal microbalance biosensor

TIRFM-QCM を用いた Amyloid βペプチドのアミロイド線維形成の動的解析

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1. Introduction

Several proteins have been identified as causative factors for neurodegenerative disorders. For example, amyloid β (Aβ) is a causative factor for Alzheimer's disease, α-synuclein causes the Parkinson disease. The common feature of these proteins is that they undergo conformational changes in some points and take highly ordered β-sheet-rich amyloid fibrils, which are frequently observed in patients. However, little is known about the specific mechanism of the amyloid fibril formed proteins assembly state. The reason is that in most of previous studies such a amyloid fibril formation is performed in the bulk solution, althought the amyloid fibril formed proteins assembly produce on the cell membrane surface. In the case of Alzheimer's disease, Aβ peptides interact with each other on the solid-liquid interfacce. Here, we have developed the flow-injection wireless-electrodeless quartz crystal microbalance (WE-QCM) biosensor combined with total internal reflection fluorescence microscopy (TIRFM) to monitor the amyloid fibril formaton of protein on the interface between solution and sensor chip in real time. We call this original technique the TIRFM-QCM.

QCM measurements observe the behavior among biochemical molecules through changes of resonance frequencies of the quartz plate. Molecular weight of biochemical molecule is one of important factors in QCM measurement, because resonance frequency of the quartz plate depends in a large part on molecular weight of biochemical molecules. Therefore, QCM measurements targeted at small molecules are difficult. To solve the ploblem, we have developed WE-QCM. The oscilator of WE-QCM is the elctrodeless AT-cut quartz palate, and thinner than that of the conventional QCM [1].

Furthermore, we developed a novel TIRFM -QCM system. It is impossible to observe images by TIRF in the oscillator of conventional QCM, because an electrode interrupt the observation of TIRF. On the other hand, our WE-QCM causes no difficulty in this issue because of perfect transparency of naked quartz [2] (**Figure 1A**).

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The TIRFM-QCM system simultaneously enables us to measure the behavior among biochemical molecules, and to observe amyloid fibril formation. Therefore, the TIRFM-QCM system can obtain unprecedented knowledge. In this study, we examined the accumulation reaction of $A\beta_{1\text{-}40}$ peptide on seeds.

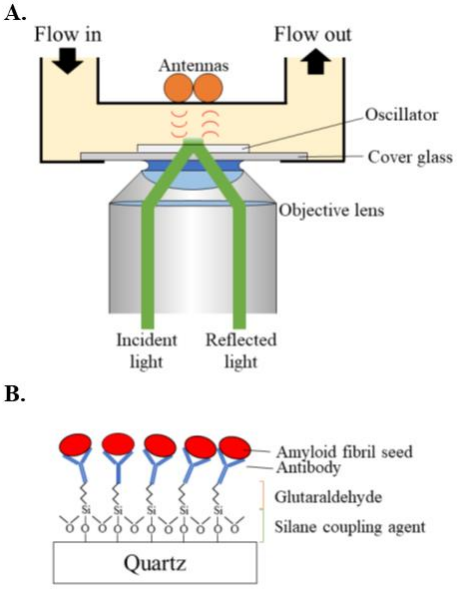

Fig. 1 (A) Schematic of cross-section view of originally developed TIRFM-QCM system. (B) Schematic of $A\beta_{1\text{-}40}$ amyloid fibril seed immobilizing method on quartz.

2. $A\beta_{1\text{-}40}$ amyloid fibril seeds immobilization

The previous immobilization method of amyloid fibril seeds with oscillator is nonspecific binding. Therefore, various conformational state of the amyloid fibril formed proteins, which are monomer, aggregation and amyloid fibril, are immobilized on oscillator. In this study, we propose an amyloid fibril specific binding method. This method immobilizese anti-amyloid fibril antibody (OC antibody) through silane coupling agent and glutaraldehyde. By means of this, $A\beta_{1\text{-}40}$ amyloid fibril seeds only can be immobilized on the oscillator (**Figure 1B**). Therefore, we can measure a pure amyloid fibril extension reaction.

3. Experimental Procedure

$A\beta_{1\text{-}40}$ peptide were dissolved in a dimethyl sulfoxide (DMSO) solution and diluted to be final concentration of 10 μM by PBS (pH 7.4) containing 100 mM NaCl. The $A\beta_{1\text{-}40}$ amyloid fibril were formed by 28 kHz ultrasound irradiation. The $A\beta_{1\text{-}40}$ amyloid fibrils were then sonicated for 1 min with a 200-kHz high-power ultrasonic generator to make seeds. A naked quartz crystal oscillator was cleaned by a UV-ozone cleaner after washing for 15 min in a piranha solution (98% H_2SO_4: 33% H_2O_2 =7:3) and rinsing with ultrapure water. The oscillator was immersed in 5% ATPES solution for 1 h at 25 °C, 5% glutaraldehyde solution for 2 h at 25 °C, and 10 μg/ml OC antibody for 3 h at 25 °C. Finally, the $A\beta_{1\text{-}40}$ amyloid fibril seeds were immobilized on the oscillator by immersing it into the seed solution for 3 h at 25 °C. The $A\beta_{1\text{-}40}$ monomer solution was diluted with PBS (pH 7.4) including 100 mM NaCl, and it was mixed with the thioflavin T (ThT) solution for the TIRFM observation. The final concentration of $A\beta_{1\text{-}40}$ peptide for flow was 10 μM. The flow rate-was 200 μl/min.

4. Results and Discussion

Figure 2 shows an example of the monitoring data by the TRIF-QCM system. The black line shows the resonance frequency change from QCM. The red line shows the ThT emission area, which was digitally calculated from TIRFM images at representative times. From this result, the resonance frequency decreased and ThT emission area increased with the flow time. Also, there seem to have correlation. The results of the previous immobilization of amyloid fibril seeds showed that increasing of ThT emission area have a lag time [2]. We think that the lag time exhibits intermediate aggregates between the monomer and the fibril. However, the result of new immobilization method didn't have a lag time. Therefore, this result show a pure amyloid fibril extension reaction. Furthermore, as **Figure 3 (A) and (B)** show, we observed amyloid fibril formation real time. From there images, amyloid fibril extension reaction are slow and need a certain amount of time.

From there results, we have succeeded in monitoring the amyloid fibril formation reaction of Aβ peptide with WE-QCM and TIRF microscopy. Also, we can measure and observe amyloid fibril formation of various conformational state and many interaction of proteins by changing immobilization method and specific antibody. Therefore, the flow-injection TIRFM-QCM system will be a beneficial tool for studying protein interaction.

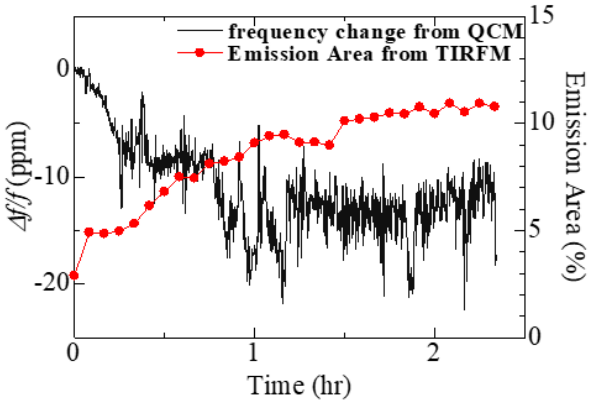

Fig. 2 Behavior of the frequency change by in injection of $A\beta_{1\text{-}40}$ monomer from WE-QCM response on $A\beta_{1\text{-}40}$ seeds and the ThT emission area ratio from TIRFM images.

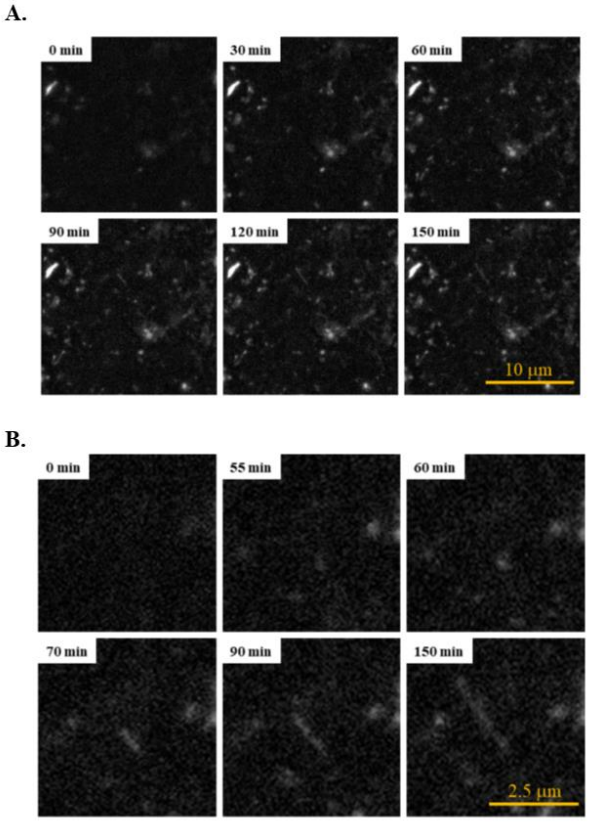

Fig. 3 (A) The TIRFM images at several stages of the 10 μM $A\beta_{1\text{-}40}$ solution-flow measurement. (B) The TIRFM images, showing the fibril extension.

References

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2. H. Ogi, M. Fukushima, K. Hamada, K. Noi, M. Hirao, H. Yagi, and Y. Goto, Sci. Rep, **4** (2014) 6960.