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Proceedings of Symposium on Ultrasonic Electronics, Vol. 35 (2014) pp. 177-178
3-5 December, 2014

Study on Nucleation-Fibrillation Dynamics of Aβ Peptides by TIRFM-QCM

TIRFM-QCM による Aβ ペプチドの核形成・線維伸長ダイナミクスの研究

Hiroki Hamada[1‡], Kentaro Noi[2], Hirotsugu Ogi[1], Hisashi Yagi[3], Yuji Goto[4], and Masahiko Hirao[1] ([1]Grad. School of Eng. Sci., Osaka Univ., [2]Inst. of Mol. Emb. and Gene., Kumamoto Univ., [3]Ctr. for Res. on Green Sustainable Chem., Tottori Univ., [4]Inst. Protein Res., Osaka Univ.)

濱田 拡規[1‡], 野井 健太郎[2], 荻 博次[1], 八木 寿梓[3], 後藤 祐児[4], 平尾 雅彦[1] ([1]大阪大院 基礎工, [2]熊本大 発生医学研, [3]鳥取大 工学部附属 GSC センター, [4]大阪大 蛋白研)

1. Introduction

Neurodegenerative diseases including Alzheimer's disease (AD) are deeply associated with assembly of specific amyloidogenic peptides. In the case of AD, amyloid β (Aβ) peptides accumulate on the cell surface in the brain, showing the neurotoxic activity. However, little is known about the specific mechanism of the Aβ assembly state. In this reaction, Aβ peptides interact with each other on the solid-liquid interface. However, in most of previous studies such an aggregation is analyzed in the bulk solution, not on a surface. 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 accumulation reaction of Aβ peptides on the interface between solution and sensor chip in real time. We call this original technique the TIRFM-QCM.

The QCM biosensor is a mass-sensitive label-free biosensor using a resonance frequency change of quartz-crystal oscillator caused by adsorption of target molecules. It monitors the deposition reaction quantitatively in real time. However, the conventional QCM biosensor has a lower sensitivity than that of other biosensors using labels such as the ELISA method. To detect lower molecular-weight molecules, a higher-sensitive QCM is necessary. To accomplish this purpose, we have developed the WE-QCM. The oscillator was drastically made thinner by excluding thick metallic electrodes and wires attached on the oscillator surfaces for generation and detection of oscillation [1].

Furthermore, we propose the TIRFM-QCM owing to the transparent electrodeless quartz oscillator. TIRF is a microscopy method with high spatial resolution due to the evanescent-light field generated by the total internal reflection of the excitation light on the cover glass surface only for the fluorescence excitation source. Not the conventional QCM but only the WE-QCM (without electrodes) allows its combination with the TIRFM.

By performing the TIRFM-QCM measurement, we can evaluate the location and quantity of interacted biomolecules simultaneously. In this study, we examined the accumulation reaction of $A\beta_{1\text{-}40}$ peptide on seeds made of $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ peptides.

2. TIRFM-QCM

The first step of AD in the aggregation process of Aβ peptide is deposition of amyloid seeds on the surface of nerve cell. Then, Aβ peptides aggregate on them to form fibrils and neurotoxic oligomers [2]. To duplicate this condition, Aβ monomers were flowed on the surface of the quartz-crystal oscillator, on which Aβ seeds were immobilized.

Figure 1 shows a schematic of the developed TIRFM-QCM sensor cell to monitor mass changes and forms of the deposition during the Aβ monomeric peptide solution flow on the electrodeless oscillator surface. Excitation light must satisfy the total reflection condition on upper surface of the oscillator where Aβ peptides aggregate. Hence, the oscillator is placed on a cover glass and held lightly by a silicon-rubber sheet, where the flow path is composed. Two line antennas are located outside the flow channel for transmitting and receiving the oscillation through electromagnetic waves contactlessly. This sensor cell enables us to measure aggregation process by WE-QCM and to observe the deposition progress by TIRF simultaneously.

E-mail: ogi@me.es.osaka-u.ac.jp

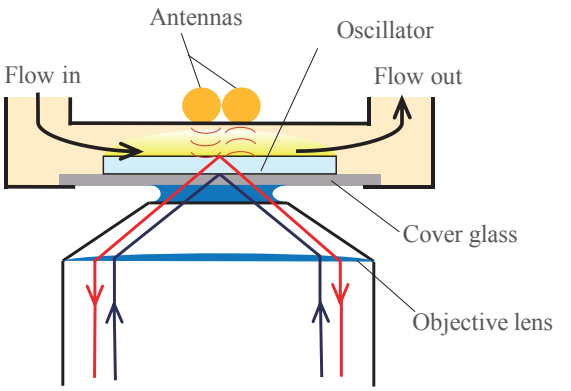

Fig. 1 Cross-section view of developed TIRFM-QCM sensor cell. One antenna oscillates the quartz oscillator and the other antenna receives the oscillation during the flow of the Aβ monomer solution.

For observing Aβ–peptides aggregations with TIRFM, we used thioflavin T (ThT) as fluorescent molecules. ThT has been adopted for evaluating nucleation and elongation of amyloid fibrils. The benzothiol-dye ThT specifically binds to the cross-β sheets constructing amyloid fibrils and produces enhanced light emission.

3. Experimental Procedure

A naked quartz crystal oscillator was cleaned by a UV-ozone cleaner after washing for 30 min in a piranha solution (98% H_2SO_4: 33% H_2O_2 =7:3) and rinsing with ultrapure water. $A\beta_{1\text{-}42}$ peptide were dissolved in a dimethyl sulfoxide (DMSO) solution and diluted to be final concentration of 50 μM by acetate buffer solution (pH 4.6) containing 100 mM NaCl. The seeds were formed by sonication at 200 MHz for 1min after stirring the peptide solution at 1200 rpm. The oscillator was immersed in the seed solution containing for 6 h at 4 °C to immobilize the seeds on it nonspecifically. The $A\beta_{1\text{-}40}$ monomer solution was diluted with ultrapure water including 100 mM NaCl, and it was mixed with the 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

Figure 2 (a) shows an example of the monitoring data by the TIRFM-QCM system. Accumulated mass on sensor chip was calculated by the resonance frequency change. The ThT fluorescence area was calculated from TIRFM images at representative times, representing the number of amyloid fibril. The deposition mass and the ThT brightness increased with the flow time. Although Aβ monomers instantaneously adhere on the sensor chip surface, it took a lag time

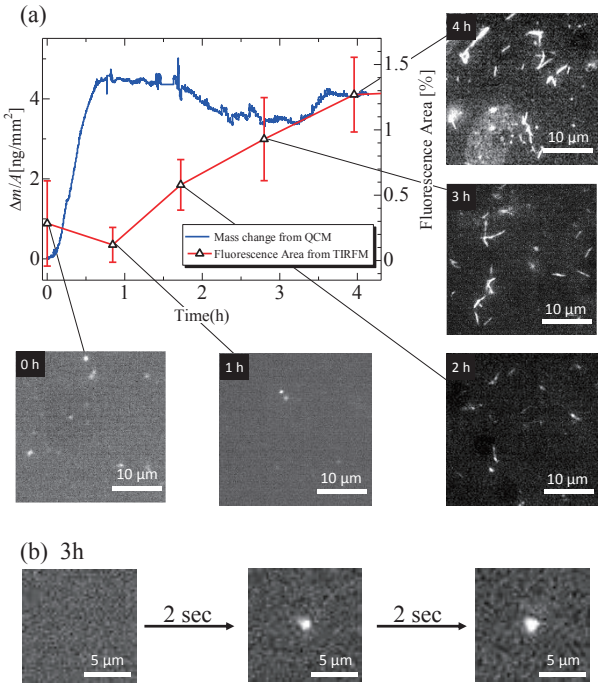

Fig. 2 (a) Deposited $A\beta_{1\text{-}40}$ monomer mass per unit area Δm from WE-QCM response on $A\beta_{1\text{-}42}$ seeds and the fibril occupation area ratio from TIRFM images. (b)The TIRFM snapshots at 3h, showing the nucleus formation.

about 1.5 h to form amyloid fibril. Furthermore, as Figure 2 (b) shows, we observed nucleation phenomenon and subsequent fibril formation in real time, which have never been observed. Thus, we have succeeded in monitoring the aggregation process of Aβ peptides on the solid-liquid interface with WE-QCM and TIRF microscopy.

5. Conclusion

From the lag time of amyloid fibril formation it is possible that intermediate aggregates between the monomer and the fibril were formed. The TIRFM images display that the β-sheet structure formation on the surface occurs very rapidly. The flow-injection TIRFM-QCM system we have developed for the first time in the world will be thus a powerful tool for studying biomolecule interactions.

References

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