

Monitoring of dissociation dynamics of Alzheimer-disease aggregates by poly-phenol with TIRFM-QCM system

ポリフェノールによるアルツハイマー病ペプチド凝集体
融解過程の TIRFM-QCM 観察

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1. Introduction

Alzheimer disease (AD) is one of the most common neurodegenerative diseases, and it is linked to a pathological polymerization of the amyloid β (A β) peptides that ultimately form aggregates on the neuron cell membrane within the human brain. However, the self-assembly mechanism of A β peptides is unsolved so that there is no effective way to cure AD primarily. AD pathogenesis has been extensively studied so as to make the toxic role of A β aggregates clear and to find a new treatment. Then, dissociation of aggregates from cell membrane can be the most effective medical approach of AD. Recently, it is reported that a poly-phenol is capable of breaking aggregates [1]. Poly-phenols are plant components deciding color, flavor, and savor. Particularly, flavonoids have strong antioxidant activity. In this study, we monitor the dissociation behavior of A β aggregates by poly-phenol visually and quantitatively. In previous studies, such a dissociation reaction was performed in the bulk solution, where interactions among aggregates govern the dissociation reaction. However, because interaction between flowing poly-phenol and deposited aggregates on solid surface occurs in vivo, we need to study the reaction for the aggregates deposited on a solid surface. So we have developed the novel instrument being capable of evaluation of amount of aggregates on the surface via flow-injection wireless-electrodeless quartz crystal microbalance (WE-QCM) biosensor combined with the total internal reflection fluorescence microscopy (TIRFM) to monitor the dissociation reaction of aggregates on the interface between solution and sensor chip by poly-phenol in real time. We call this original technique the TIRFM-QCM.

The QCM biosensor is an acoustic device which detects mass change on the surface of quartz

crystal without labeling by detecting resonance frequency change of the quartz crystal. However conventional QCM is low-sensitive because it has thick metallic electrodes and wires attached on the oscillator surfaces. For the reason mentioned above, we developed the WE-QCM, where electrodes and wires were excluded [2]. The oscillator gets thinness dramatically, and it can detect interactions between low molecular-weight molecules.

Furthermore, we integrated the WE-QCM in TIRFM [3]. The oscillator of the WE-QCM is a naked quartz and transparent for wide-range wavelength: The WE-QCM is easily combined with TIRFM. TIRFM is a visual assessment device with high spatial resolution using the evanescent-light field generated by the total internal reflection of the excitation light on the cover glass surface, allowing to provide fine images with low background light.

In this study, we monitor the dissociation process of A β aggregates by poly-phenol anthocyanin, delphinidin-3-galactoside with the TIRFM-QCM.

2. TIRFM-QCM

Figure 1 shows a schematic illustration of the TIRFM-QCM sensor cell. The anthocyanin solution flows in and out through flow path from injection holes. For the total reflection condition on upper surface of the oscillator, the oscillator was placed on the cover glass and held lightly by the silicon-rubber sheet, where the flow path is composed. Twin antennas are set outside the flow path, and they transmit and receive the resonance oscillation through electromagnetic waves. This sensor cell enables us to evaluate the amount of removed aggregates from the surface by the WE-QCM and to observe its dynamics visually by TIRFM simultaneously.

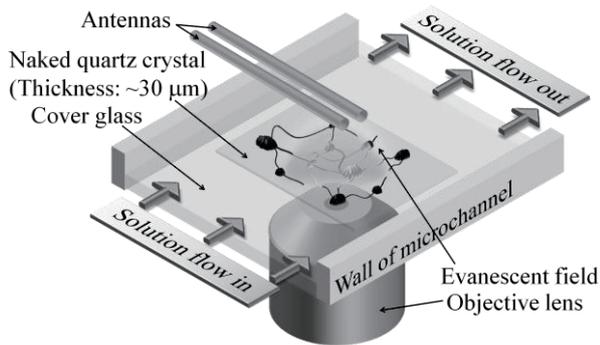


Fig. 1 Schematic illustration of developed TIRFM-QCM sensor cell. One antenna oscillates the quartz oscillator with constant resonance frequency, and the other antenna receives the resonance frequency through electromagnetic waves while solution flows. White part is visible and black part is invisible by TIRFM, however quantitative change of the mass on the all over the oscillator surface can be detected by the WE-QCM.

For sighting the dissociation of aggregates with TIRFM, we used fluorescent label thioflavin T (ThT). ThT distinguishes only fibril-related aggregates and produces fluorescence. That's because it only binds to the particular structure of aggregates and generates enhanced light emission. In this study, we used oscillator quartz crystal chip of $28.5 \mu\text{m}$ thick, corresponding to the resonance frequency of 58 MHz.

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. Firstly, we formed aggregates on the surface of oscillator by the same procedure as described previously [4]. Briefly, $10 \mu\text{M}$ $\text{A}\beta_{1-40}$ monomer solution including $30 \mu\text{M}$ ThT was injected to $\text{A}\beta_{1-42}$ -nuclei immobilized oscillator surface. Secondly, we injected $300 \mu\text{M}$ anthocyanin solution containing $30 \mu\text{M}$ ThT to the aggregates formed on the oscillator at room temperature. The flow rate was $200 \mu\text{l}/\text{min}$.

4. Results and discussion

Figure 2 shows example data obtained by the TIRFM-QCM system. Adsorbed quantity on the oscillator was calculated from change of resonance frequency. We found that the thin fibrils were dissociated almost within 1 h by flowing the anthocyanin solution: Such a dramatic dissociation ability of anthocyanins is first confirmed in this study. The large aggregates such as seeds and thick fibrils remained longer, however they were

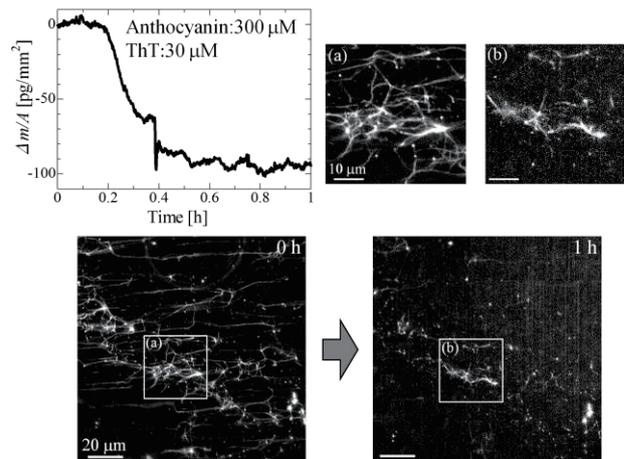


Fig. 2 The time course of mass per unit area on the surface measured by WE-QCM and fluorescence images at same point by TIRFM in dissociation process as flowing poly-phenol anthocyanins solution.

dissolved in 15 h. The WE-QCM indicates that the aggregates on the oscillator surface decreased with 1 h, being consistent with the TIRFM observation. Thus, we confirm great dissociation ability of poly-phenol anthocyanins by the TIRFM-QCM system.

5. Conclusion

Surprisingly, poly-phenol anthocyanins, delphinidin-3-galactoside have very strong dissociation ability against aggregates. Furthermore, poly-phenol anthocyanins are possible to dissociate not only thin fibrous aggregates, but also seeds and thick aggregates. These findings will contribute to understand the dissociation mechanism of $\text{A}\beta$ aggregates by anthocyanins.

References

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