2P2-6

Effect of anthocyanin to dissolve Aβ amyloid fibrils studied by TIRFM-QCM biosensor.

TIRFM-QCM を用いた Aβ アミロイド線維に対する
アントシアニンの融解効果の研究

Kohei Kamada[1†], Kentaro Noi[1], and Hirotsugu Ogi[1] ([1]Grad. School Eng., Osaka Univ.)
鎌田　航平[1†]，野井　健太郎[1]，荻　博次[1] ([1]阪大院・工)

1. Introduction

Alzheimer's disease (AD) is the most common dementia. In 1907, Alois Alzheimer described pathological alterations in the brain of a patient of dementia which was named Alzheimer's disease. Despite the fact that intensive efforts have been made by many researches, there is still no effective method of treatment for AD. Many scientists and medical doctors recognize that deposition of amyloid β (Aβ) peptides and their aggregation on neurons cause AD. Aβ takes several phases, including monomer, oligomer, and fibril. Especially, Aβ amyloid fibril is always confirmed at AD patients' neuron. As a candidate method for AD treatment, some researches focus on dissolving Aβ amyloid fibril. It has reported that some of anthocyanins (a kind of polyphenol) can inhibit the fibril formation in the bulk solution.[1] However, there is no such a report on a solid surface, closer to surroundings in vitro, and there is no systematic study on anthocyanin's ability to dissolve Aβ amyloid fibril. To monitor formation of Aβ amyloid fibrils and their dissolving process by anthocyanin on the solid surface, we use the TIFM-QCM assay system. It is the combination of the total internal reflection fluorescence microscopy (TIRFM) to monitor the change in the fibril structure and wireless-electrodeless quartz crystal microbalance (WE-QCM) biosensor to monitor the change of amount of Aβ molecules on the surface. Here, we use delphinidin 3-galactoside (DP3G: a kind of anthocyanin) for dissolving Aβ amyloid fibrils.

2. TIRFM-QCM Biosensor

Fig.1 shows illustrates the TIRFM-QCM biosensor measurement. The WE-QCM device is set on the objective lens of TIRFM. The flow channel is set on a cover glass, along which the $A\beta_{1\text{-}40}$ solution or the anthocyanin solution flow. The quartz oscillator is put in the middle of the flow channel, whose vibration is excited by the antenna for transmission by applying alternating electric field, and the vibrational signals are detected by the other antenna through the piezoelectric effect.

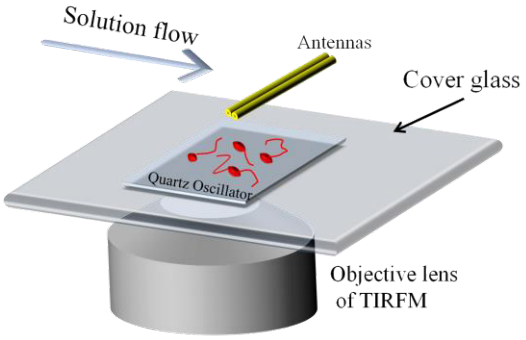

Fig.1 Schematic illustration of TIRFM-QCM biosensor.

TIRFM is a type of fluorescence microscopes. The incident light is totally reflected on the surface of quartz oscillator and then the evanescent light is generated. TIRFM uses the evanescent light as the excitation light, so that the fluorescence images become dramatically clear because of the absent of the scattered excitation lights. It is effective to observe the change in the fibril structure by adding thioflavin T (ThT) molecules in the flowing solution because ThT emits strong light when it binds with the β-sheet structure of fibrils. Because biosensor chips generally have metal coatings on their surfaces, it is difficult to perform the TIRFM observation on such chips. The WE-QCM chip has no coating, thus, we can use the WE-QCM for TIRFM. This is the first assay system which achieves combination of QCM and TIRFM. Furthermore, it has high mass sensitivity because the sensitivity of the QCM deteriorates with the heavy metallic coatings.

3.Experimental Details

3.1 Monitoring formation of Aβ amyloid fibril

We irradiated the $A\beta_{1\text{-}42}$ amyloid-fibril solution with ultrasound to break the fibrils down into fragments. They were fixed on the quartz oscillator as seeds for Aβ amyloid fibril. After the

Email: Ogi@prec.eng.osaka-u.ac.jp

resonant frequency of WE-QCM became sufficiently stable while flowing buffer solution (ultra pure water with 30 μM ThT), we let the water solution including 10 μM $A\beta_{1\text{-}40}$ peptides and 30 μM ThT flow over the seeded quartz oscillator and monitored formation of Aβ fibril for 10 h.

3.2 Monitoring Aβ fibril Dissolving process

After forming the $A\beta_{1\text{-}40}$ fibrils on the quartz surface, we let the water solution including 80 μM DP3G and 30 μM ThT flow over the quartz oscillator with the fibril and monitored the dissolving process of Aβ amyloid fibril for 10 h.

For a control experiment, we let the water solution including 80 μM chlorogenic acid hemihydrate (a type of polyphenols) and 30 μM ThT flow over the oscillator for 10 h.

4. Results and Discussion

Fig.2 shows the fluorescent images by TIRFM during formation of Aβ amyloid fibrils. A few hours later, the Aβ amyloid fibril emerged on the surface of the oscillator. We thus succeed in forming Aβ amyloid fibrils, which agrees with our previous study[2].

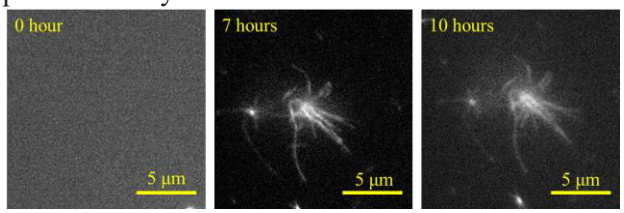

Fig.2 The fluorescence images by TIRFM in forming Aβ amyloid fibril. Left shows the image when the flow $A\beta_{1\text{-}40}$ solution is injected (we set this time to be 0 h). Middle shows an image when the amyloid fibril appeared. Right shows the image at the end of the measurement.

Fig.3 shows the resonance frequency change measured by the WE-QCM during the flow of DP3G, and **Fig. 4** shows the corresponding fluorescent images taken by TIRFM. From Fig.3, the frequency increase indicates the decrease of mass on the surface of the quartz oscillator. The increase of about 30 ppm means the decrease of about 2 pmol of $A\beta_{1\text{-}40}$ monomers from the surface. From Fig.4, the light lines become weak and then disappear. Therefore, DP3G seems to have ability to dissolving Aβ amyloid fibril.

Fig.5 shows the fluorescent images by the TIRFM of the control experiment by the chlorogenic acid hemihydrate, where we clearly see the Aβ amyloid fibril even after the flow of the control anthocyanin for 10 h. These images significantly differ from those in the DP3G experiment. Thus, it is obvious that DP3G has a strong effect to dissolve Aβ amyloid fibril.

Therefore, we succeeded to analyze the forming and dissolving Aβ amyloid fibril from two different viewpoints, mass changes and fluorescence imaging, by TIRFM-QCM. We will make experiments for other anthocyanins to evaluate their Aβ-fibril dissolving ability, and investigate its correlation with the number and position of hydroxyl group in the anthocyanin molecule.

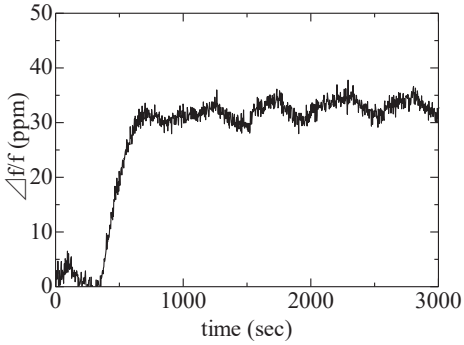

Fig. 3 The resornance frequency change by WE-QCM of dissolving Aβ amyloid fibril by DP3G. Time=0 means the time when stareted to flow DP3G solution.

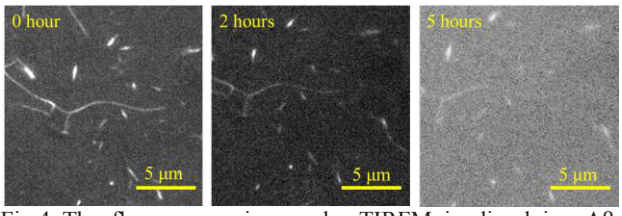

Fig.4 The fluorescence images by TIRFM in dissolving Aβ amyloid fibril. The left image is the image when time of starting to flow DP3G (0 h). The middle one is the image when started dissolving the amyloid fibril. The right one is the image 5 h later . Then, fibril almost disappeared.

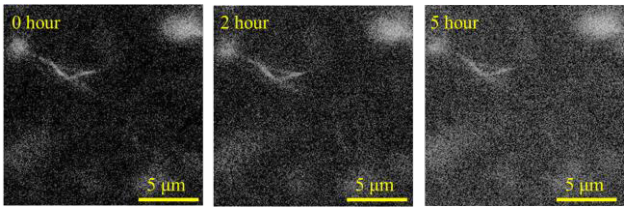

Fig.5 The fluorescence images by TIRFM in a control experimental. 5 h later, the fibril had almost no change.

References

1. H. Iwasa, H. Kameda, N. Fukui, S. Yoshida, K. Hongo, T. Mizobata, S. Kobayashi and Y. Kawata: Biochemistry **52** (2013) 9202.

2. H. Hamada, H. Ogi, K. Noi, H. Yagi, Y. Goto, and M. Hirao: Jpn. J. Appl. Phys. **54** (2015) 07HE01.