3E2-3

Proceedings of Symposium on Ultrasonic Electronics, Vol. 37 (2016)
16-18 November, 2016

Viscoelasticity response during fibrillation of amyloid β peptides on quartz crystal microbalance biosensor
QCM バイオセンサーによるアミロイド β ペプチドの線維化変遷に伴う粘弾性挙動の研究

Yen-Ting Lai[1†], Hirotsugu Ogi[1], Arihiro Iwata[1] and Masahiko Hirao[1] ([1]Grad. School Eng. Sci., Osaka Univ.)
Yen-Ting Lai[1†]、荻博次[1]、岩田有弘[1]、平尾雅彦[1] ([1]大阪大　基礎工)

1. Introduction

Amyloid β (Aβ) peptide is an insoluble fibrous protein, which is resulted from cleaving the amyloid precursor protein (APP) by α or β secretase [1, 2]. It is recognized that Aβ peptides are related to Alzheimer's disease (AD) through their neurotoxic aggregates, which are intermediates in the conformational transition from monomer to nuclei and then to fibril [3]. Owing to the process, the mechanical property of Aβ aggregates should vary while the process concerns structural deformation (fibrillation) and particle deposition (accumulation). Therefore, the understanding of the variation can be applied to dedicated diagnosis of Alzheimer's disease.

As Aβ aggregation exhibits structural change such as oligomer to fibril, the viscoelasticity property is considered to be fluctuated. Here, we present the quartz crystal microbalance (QCM) sensor with high fundamental resonance frequency (~58 MHz), without wires and electrodes (wireless electrodeless, WE-QCM), packaged in microchannel [4] as shown in **Fig. 1** to perform the real-time monitoring of Aβ aggregate. In previous study, Kanazawa *et al.* found that viscoelastic parameters such as viscosity and shear modulus affect the resonance frequency shift [5]. It is also proved that the sensitivity and the intensity of the sensor would be diminished with electrode coatings on quartz surface because of very large inertia effect of electrodes and attached wires. Therefore, the QCM is designed with electrode-free surfaces with antennas replaced over the quartz with spacing. By applying the dynamic electric field, the quartz can still be excited piezoelectrically [4].

It is now believed that certain misfolded aggregates (known as "seeds") can capture other Aβ molecules to grow the misfolded oligomeric form. In this work, aggregation reaction on different seeds, $Aβ_{1-40}$ and $Aβ_{1-42}$ is investigated. The monomer, $Aβ_{1-40}$, is used as the target because of its highest quantity in human brain; we thus study the homogenous-binding of $Aβ_{1-40}$ to $Aβ_{1-40}$ seed and the cross-binding of $Aβ_{1-40}$ to $Aβ_{1-42}$ seed. By measuring four overtones, the viscoelasticity behavior is inversely characterized [6]. Moreover, the QCM surface is scanned with atomic-force microscope (AFM) to verify the corresponding phases.

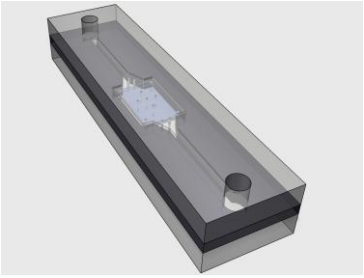

Fig. 1 the 3D scheme of quartz embedded RAMNE-Q.

2. Experiment

The binding behaviors of $Aβ_{1-40}$ monomer to $Aβ_{1-42}$ seeds and that to $Aβ_{1-40}$ seeds are monnitored under 24 °C in vitro, where the seeds are immobilized as receptor and the monomers are flowed as target. The experiments were done 2 to 3 times to confirm reproducibility.

2-1 Solution preparation

For preparing $Aβ_{1-42}$ seed, the monomer was dissolved with dimethyl sulfoxide (DMSO) with magnetic stirring for 10 min with 150 rpms and was diluted in acetate buffer solution (ABS, pH = 4.6) into 50 μM at 1:19 for DMSO to ABS. In case of $Aβ_{1-40}$ seed, it was diluted in PBS. The seed solutions were then stirred for 24 h at 1200 rpm. Afterwards, the solutions were ultrasonified under 230 kHz to fragmentate the aggregates into Aβ seeds.

The target, $Aβ_{1-40}$ monomer, was dissolved with DMSO under 10 min of magnetic stirring and was diluted by ultrapure water into 15 μM under

ogi@abc.me.es.osaka-u.ac.jp

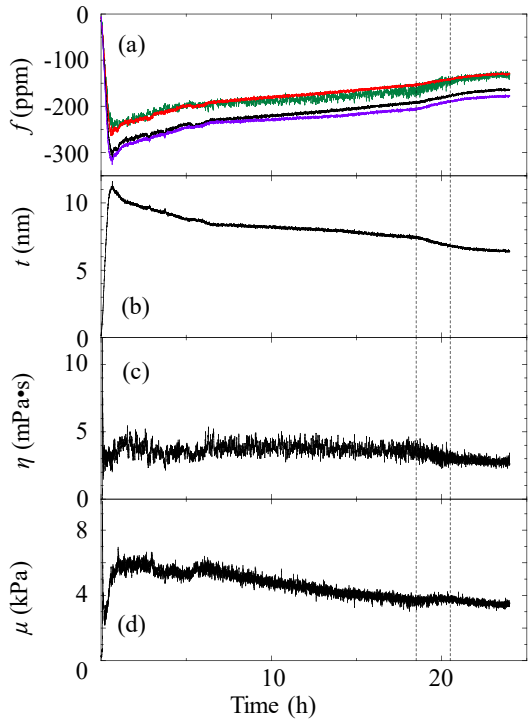

Fig. 2 The frequency shift of (a) cross-binding, for blue: fundamental mode; black: 3rd tone; red 5th tone; green: 7th tone. And the (b) protein thickness, (c) viscosity, and (d) shear modulus inverse calculated.

coherent ratio of 1:20 for DMSO to ultrapure water.

2-2 Sensor preparation

The bare quartz chip of 1.7 mm x 2.5 mm cross-section with 28 μm thick was applied for sensing. The chip was first washed by piranha solution (7:3 of H_2SO_4 to H_2O_2), rinsed by ultrapure water and cleaned with UV-ozone cleaner for 15 min. Next, the seeds were immobilized nonspecifically onto the quartz chips for 18~20 h. The chip was then set into sensor cell.

2-3 Experiment procedure

First, ultrapure water was flowed over the sensor surfaces, as the buffer liquid in this experiment. After the frequency became stable, the target solution was injected and flowed for 24 h. The 1st, 3rd, 5th, and 7th overtones were simultaneously monitored by network analyzer.

3. Result and discussion

The measurement result of the cross binding case is shown in **Fig. 2 (a)**. With the inverse calculation, evolutions of protein layer thickness (t), viscosity (η), and shear modulus (μ) were

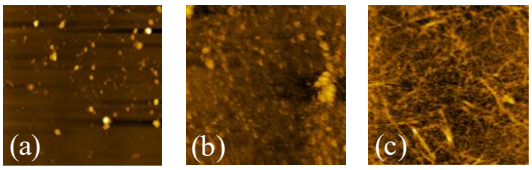

Fig. 3 The AFM figures (a) before injection, (b) before the ramp (~17 h), and (c) after the ramp (~22 h). All figures are taken under 5 μm x 5 μm.

determined as in **Fig. 2 (b)-(d)**. First, the frequencies decrease by 350 ppm for 1 h after the injection because of binding reaction between monomer and seeds. Here, the increment in t to 12 nm has confirms the binding. The frequencies then slowly increase with constant rates, and they have experienced the ramp near 18 h and the situation have stood for about 2 h. During the period, the η and μ vary in reverse direction.

Fig. 3 shows AFM images before injection, just before the ramp, and after the ramp. Before the injection, we see the immobilized seeds, and before the ramp, many amorphous-like structures, indicating the deposition of Aβ peptides. After the ramp, we see significant fibrils over the sensor surfaces, indicating the transition from oligomer to fibrils. The ramp is then characterized as the structural transition of the aggregate. The decrease in η during the ramp can be explained as weaker overall binding due to the contact face limitation of fibrillated oligomers to the seeds. The increase in μ can then be interpreted as the stiffness increase caused by transform from oligomers into fibrils.

4. Conclusion

This analysis proves the possibility of QCM evaluating of Aβ aggregation, and can be a good dedication in real-time monitoring of its mechanism.

References

1. D. J. Selkoe, M. B. Podlisny, C. L. Joachim, E. A. Vickers, G. Lee, L. C. Fritz, and T. Oltersdorf: *Proc. Natl. Acad. Sci. U.S.A.* **19** (1988) 7341.
2. J. M. Nussbaum, M. E. Seward and G. S. Bloom: *Prion* **7** (2013) 14.
3. T. Lührs, C. Ritter, M. Adrian, D. Riek-Loher, B. Bohrmann, H. Döbeli, D. Schubert and R. Riek: *Proc. Natl. Acad. Sci. U.S.A.* **102** (2005) 17342.
4. H. Ogi: *Proc. Jpn. Acad.* **B 89** (2013) 401.
5. K. K. Kanazawa and J. G. Gordon II: *Anal. Chmi. Acta.* **175** (1985) 99.
6. T. Shagawa, H. Torii, F. Kato, H. Ogi and M. Hirao: *Jpn. J. Appl. Phys.* **54** (2015) 096601.