Proceedings of Symposium on Ultrasonic Electronics, Vol. 36 (2015)
5-7 November, 2015

Detection of target protein via aptamer electrostatically immobilized on wireless-electrodeless QCM biosensor chip

無線無電極QCMを用いたバイアス電位によるアプタマーの固定化と標的蛋白質検出

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1. Introduction

Aptamers attract our attentions as drug materials that replace with antibodies, because they have many advantages over antibodies. Aptamer is a single stranded oligonucleotide or peptide molecule and takes a characteristic form by means of intermolecular interaction between complemental pairs. Using their unique conformations, aptamers specifically recognize and bind to biomolecules, even to those that antibodies fail to attach. They can remain their structures at elevated temperatures and low pHs. Moreover, the most noticeable advantage is that they can be synthesized at low cost. The methodology to select possible aptamers for target proteins is established, called SELEX[1] (systematic evolution of ligands by exponential enrichment). It is then necessary to validate the specificity of the selected aptamers as a drug. However, measuring the dissociation constant K_D remains a problem to be solved for aptamers because they often charge negatively.

Biosensors play important roles in biomedical fields; for example in diagnosis biosensors are used to detect biomarkers, which reach high blood levels when corresponding diseases develop. Biosensors also contribute to the drug-discovery process to evaluate specificity of candidates to target materials. Biosensors were attempted to apply to aptamers, but it was difficult because of the negative charge, which prevents them from approaching negatively charged sensor chips.

To investigate affinity between aptamers and proteins, label-free biosensors are preferable. Fluorescence analysis, such as well-known enzyme-linked immunosorbent assay (ELISA), is unusable. Furthermore, strong negative charge of aptamers makes many biosensors inapplicable, including surface plasmon resonance (SPR), because of the negative charge of the sensor chips. Then, we propose a new method for investigating interactions between aptamers and proteins by applying the wireless-electrodeless QCM (WE-QCM).

QCM is a mass detecting biosensor. The more mass loading accumulates on the quartz resonator, the more its resonance frequency changes. QCM is a label-free biosensor and we can measure interactions between biomolecules in less steps than biosensors using labels. Measuring the frequency shifts in real time, the affinity between the immobilized biomolecules and the targets flowed with solution is obtained. QCM has many advantages but has some drawbacks. The most important one is its sensitivity. Conventional QCM has gold electrodes on the both surfaces of the quartz crystal to resonate it. To supersensitize QCM we need to reduce the weight of quartz by thinning it, but the inertia resistance caused by the gold electrodes interfere the vibration of quartz resonator. Thus the gold electrodes make it difficult to supersensitize QCM. We then removed them from both surfaces of the quartz crystal and developed the WE-QCM[2].

We apply an external electric field by the set of transceiving antennas and measure the resonance frequency of the quartz oscillator contactlessly. We achieved ultrasensitive QCM by thinning the quartz crystals. Taking advantage of this naked quartz resonator, we propose a new strategy for detecting aptamer-protein bindings by the WE-QCM. We had quartz resonator surface positively charged by applying electrostatic pressure and measured the resonance frequency. Since aptamers have strong negative charge, we immobilized them directly on the oscillator without using any linkers. Injecting target proteins after the immobilization, we can detect aptamer-protein bindings. Heparin-binding EGF-like growth factor (HB-EGF) is highly expressed in the various cancer cells and has been studied as a target protein for drug delivering system[3]. In this paper we use the aptamers which

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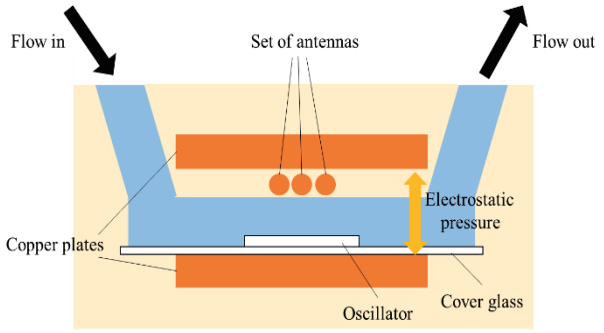

Fig. 1 Experimental Schematic of contactlessly measuring the resonance frequency of the quartz oscillator with applying the electrostatic pressure contractually by the copper plates.

bind to the HB-EGF for investigating the availability of this new strategy.

2. Experimental procedure

We used HB-EGF binding RNA aptamers (116mer, MW=35,720). The buffer solution was 40 mM HEPES, 200 mM NaCl, 20 mM $NgCl_2$, and 100 mM KCl. Before use, the aptamers were heated at 95 ºC for 3 min in the incubator, cooled for 10 min in the ice, and then incubated at 37 ºC for 15 min. After these steps, the aptamers were diluted into intended concentration with the buffer solution.

We used an AT-cut quartz with fundamental resonance frequency of 60 MHz. Before setting in the sensor cell, the sensor chip was immersed in the piranha solution (H_2O_2:H_2SO_4 = 3:7 in volume), rinsed with ultrapure water, and irradiated with ultraviolet light. Then, we set the sensor chip in the sensor cell as shown in Fig. 1. The sensor chip was placed on the cover glass, beneath which a copper plate was attached and linked to the positive side of the DC power source. Another copper plate is present upper of the sensor cell, and it was linked to the negative side. With this pair of copper plates, the electrostatic pressure was applied and the upper surface of the sensor chip was charged positively. By means of the set of transceiving antennas, the vibration of the quartz oscillator was excited and the response from the WE-QCM was obtained contactlessly.

In this paper we discuss about the result of immobilizing the aptamers on the sensor chip with and without electrostatic pressure. The distance between the copper plates was ~3 mm and the electrostatic pressure was 100 V, giving more than 30 V/mm. We prepared the aptamer solution with the concentration of 1 μM.

3. Results and discussions

We show the example of the results of flowing the aptamer solution with and without electrostatic

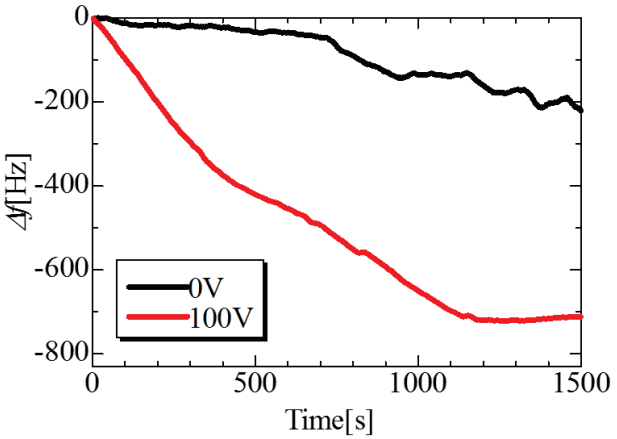

Fig. 2 Comparison lines about frequency shifts by immobilizing aptamers on the sensor chip. Black line shows the result of not applying electrostatic pressure and Red line shows that of applying electrostatic pressure.

pressure in Fig. 2. Without electrostatic pressure, the resonance frequency changed little and took a long time to be saturated. This indicates that the aptamers were immobilized on the quartz chip nonspecifically. On the other hand, with the electrostatic pressure, the frequency shifts were significantly larger. Moreover what is noteworthy is that it requires a much shorter time to be stable, even compared to antigen-antibody interactions. This indicates that aptamers are attracted on the sensor chip by the electrostatic force with high affinity. After aptamers immobilization by electrostatic pressure, it is expected to detect aptamer-protein bindings flowing the proteins. Thus the new strategy for detecting proteins via aptamers electrostatically immobilized on the WE-QCM is expectable.

4. Conclusion

In this study, we proposed the new strategy for detecting aptamer-protein bindings. We designed to immobilize aptamers on the oscillator via electrostatic pressure and investigate aptamer-protein bindings. In fact, the difference of the WE-QCM responses with applying and without applying electrostatic pressure was clearly shown. Applying electrostatic pressure, aptamers were effectively immobilized on the sensor chip. Then flowing target proteins, it is expected to detect target proteins by aptamers immobilized on the WE-QCM. Coupling this strategy with SELEX can contribute to drug development.

References

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