Proceedings of Symposium on Ultrasonic Electronics, Vol. 31 (2010) pp. 69-70
6-8 December, 2010

Detection of C-Reactive Protein with Mass-Amplified Sandwith Assay using Electrodeless Quartz-Crystal Microbalance Biosensor

無電極水晶振動子バイオセンサによる質量増感サンドイッチ法を用いたC反応性タンパク質の検出

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1. Introduction

The piezoelectric quartz crystal microbalance (QCM) is a mass-detectable biosensor, which can detect biomolecules adsorbed to the corresponding receptor protein immobilized on the surface of the quartz oscillator through the change in the resonance frequency of the oscillator. It allows to detect diagnostic protein markers, such as C-reactive protein (CRP) for inflammation [1] and carcinoembryonic antigen (CEA) for digestive organ cancer [2]. Their detection in the early stage contributes to effective treatments for the diseases, and higher sensitive biosensors are required.

The mass sensitivity of the QCM biosensor can be improved by thinning the quartz crystal. We then propose a wireless-electrodeless (WE) technique and achieved advanced QCM biosensors using much thinner quartz oscillators than those used in conventional QCMs by a factor of 0.03, showing fundamental frequencies up to 180 MHz [3-6]. (Corresponding mass sensitivity is improved by three orders of magnitudes.)

In this study, we apply the WE-QCM to the high sensitive detection of CRP to demonstrate its ability as a diagnostic tool. However, there has been a problem in a label-free assay that human serum contains much larger amount of other proteins as well as the target protein. They are adsorbed to the sensor surface nonspecifically and decrease the resonance frequency, being comparable or exceeding the contribution of the target protein to the frequency change. Then, we propose a mass-amplified sandwich assay, where the target protein is sandwiched between the primary antibody immobilized on sensor surface and mass-amplified second antibody; the frequency change can be enhanced by mass addition. In this study, we use biotinated second antibody with streptavidin molecules for the mass amplification (**Fig. 1**).

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2. Preparation of the sensor chip

The 9-μm thick AT-cut bare quartz plate, whose fundamental resonance frequency is 182 MHz, was used. Many proteins are directly adsorbed to the naked quartz surface [5,6], and utilizing this binding ability, the primary anti-CRP was immobilized on the quartz surface as follows. The crystal was cleaned in piranha solution (98% H_2SO_4 : 33% H_2O_2 = 7 : 3). After rinsing with ultrapure water, it was immersed in phosphate buffered saline (PBS) solution (pH 7.4) containing 500 μg/ml primary anti-CRP antibody for 5 h at 4ºC and rinsed by the PBS solution.

To prepare the mass amplification solution, second anti-CRP antibody was biotinated using Dojindo biotin labeling kit. The biotin with the active ester group was mixed in the second anti-CRP antibody solution and incubated for 30 min at 37ºC. To remove the unreacted biotin the mixture was centrifuged and the biotinated anti-CRP antibody was obtained. Then, 1 μg/ml

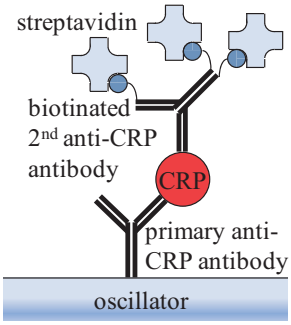

Fig. 1 Schematic of the mass-amplified sandwich assay. the weight of the 2nd antibody is increased by streptavidin molecules via biotin terminals.

biotinated anti-CRP antibody and 10 μg/ml streptavidin are mixed in the PBS solution and stirred for 1 h before injection.

3. Experiment

The sensor chip was set into the homebuilt flow injection system for continuous and stable monitoring of the resonance frequency. The temperature in the cell was maintained at 37±0.1ºC. The carrier solution was PBS, and the flow rate was 500 μl/min. After the resonance frequency was stable during the PBS flow, the CRP solution was injected, which was followed by the injection of the mass-amplified second anti-CRP antibody solution.

4. Results and Discussion

Fig. 2 shows the represent frequency response through the experiment. As shown **Fig. 2**, the small frequency change by the injection of the 1 ng/ml CRP/PBS solution was significantly amplified by the injection of the mass-amplified second anti-CRP antibody solution. **Fig. 3** shows the comparison of the frequency responses for injections of the mass amplification solution following injections of 0.1-100 ng/ml CRP/PBS solutions, showing good correlation between the frequency change and the concentration of the analyte. 'Nonspecific' in **Fig. 3** denotes the frequency response observed when the antigen CRP solution was not injected, indicating the nonspecific binding of the second antibody molecules. Because the frequency decrement in the nonspecific case was smaller than that of specific responses, specificity of the mass-amplified sandwich assay is confirmed.

We succeed in detecting 0.1-100 ng/ml CRP,

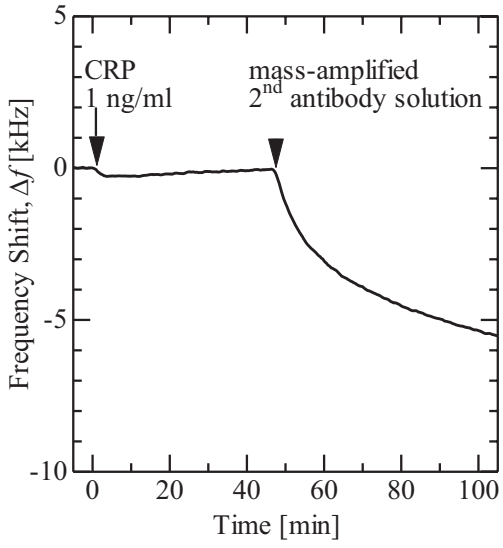

Fig. 2 Frequency change behaviors for the injection of 1 ng/ml CRP solution and the mass-amplified 2nd antibody solution.

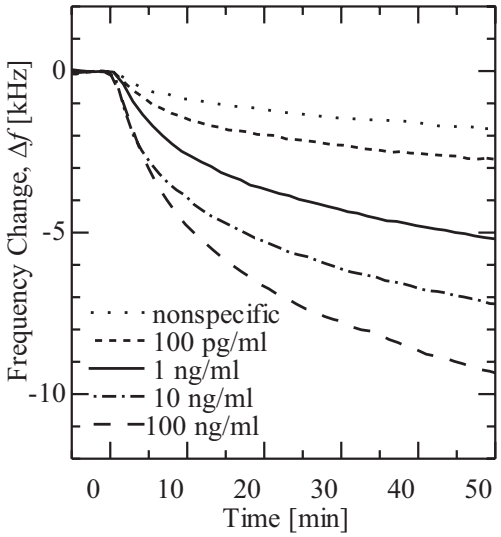

Fig. 3 Frequency changes caused by injections of the mass-amplification solution after injections of various concentrations of CRP solutions.

and this value was enough smaller than the threshold level of CRP (30 ng/ml) in the serum of a healthy adult.

5. Conclusion

We developed a high-frequency WE-QCM biosensor with the fundamental frequency of 182 MHz, and detecting CRP with the mass-amplified sandwich assay. Using this method, a low concentration antigen which could not be detected by the direct detection can be detected. We establish WE-QCM for applications in diagnosis by mass-amplified sandwich assay.

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