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High-sensitive detection of C-reactive protein by oriented antibody using RAMNE-Q biosensor

ラムネ水晶振動子バイオセンサを用いた配向抗体による C 反応性プロテインの高感度検出

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1. Introduction

The quartz crystal microbalance (QCM) is used as a biosensor, which allows real-time monitoring, quantitative analysis, and label-free measurement[1-3]. The QCM biosensor is a mass detection type sensor, which detects the loading mass as the frequency shift when the analyte is selectively captured with receptors such as antibodies immobilized on the surface of the quartz plate vibrating at the resonance frequency. As the sensitivity breakthrough for such a QCM biosensor, we developed the resonance acoustic microbalance with naked-embedded quartz (RAMNE-Q) biosensor, which has the high-frequency quartz resonator (up to ~200 MHz) packaged without fixing mechanically in the silicon microchannel fabricated by the micromachining technology[4, 5].

In this study, we demonstrate high-sensitive detection of C-reactive protein (CRP), which increases due to the infection and inflammation in humans, by applying oriented anti-CRP receptor layer using the RAMNE-Q biosensor into which installed the high-frequency quartz resonator (172 MHz). We focus particularly on high affinity between the protein molecules and the surface of the blank quartz crystal in this study. Concretely, the streptococcal protein G (SPG) molecules are first immobilized on the naked quartz surfaces nonspecifically, and then anti-CRP molecules, which form the receptor layer, are flowed, which is expected to bind to the SPG molecules with suitable orientation to capture the CRP molecules efficiently. In this study, the detection of 100 pg/ml CRP solution through a series of biomolecular reaction is displayed. Moreover, the affinity parameters are identified from the real-time measurement.

2. Experimental Procedure

The RAMNE-Q chip (**Fig. 1**) is installed in the measurement cell, which is equipped with two pipes and made of Teflon. This cell has two copper plate antennas to operate the quartz resonator wirelessly in the microchannel of the chip[5].

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The tone-burst signal of the electromagnetic wave is impressed from the generation antenna to the quartz resonator, which excites the vibration by the inverse piezoelectric effect. Afterwards, the potential difference is generated by the piezoelectric effect at the vibration, which emits electromagnetic wave to be detected by the detection an antenna.

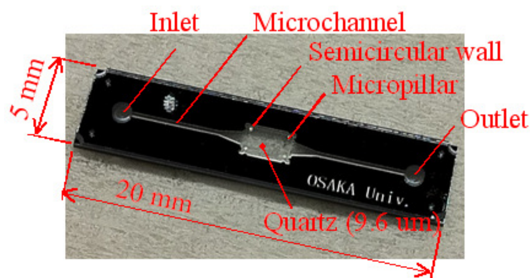

Fig. 1 Appearance of RAMNE-Q chip, which was fabricated by MEMS technology.

The procedure of the assay with the orientated antibody is shown in **Fig. 2**. The flow rate (250 µl/min) was controlled constantly by the liquid chromatography (SCL-10A and DGU-20A3, Simazu, Japan). Before all experiments, the washing procedure was executed by flowing isopropyl alcohol in the microchannel for 30 minutes to remove the proteins in the state that installed the RAMNE-Q chip in the measurement cell. And 1 mg/ml bovine serum albumin (BSA) was then flowed into the measurement system without the RAMNE-Q chip to block pipes inside from the nonspecific adsorption of the proteins. After blocking procedure, the phosphate buffered saline (PBS) as the buffer solution was flowed in the course in which solution is poured. The PBS buffer solution was flowed in the microchannel after attaching the RAMNE-Q chip again, and after the baseline became stable within 1 ppm frequency fluctuation, the SPG solution (0.3 mg/ml in PBS) was injected to immobilize the SPG molecules nonspecifically on both sides of the quartz surfaces, and then the surfaces were rinsed with the buffer solution flowed in the microchannel. Afterwards, 0.2 mg/ml the anti-CRP solution was injected to construct the oriented receptor layer through the specific binding between the SPG molecule and Fc

region of the anti-CRP molecule. The CRP solution (100 pg/ml in PBS) was then injected to detect the binding reaction. After the injection sequence above, the quartz surfaces were rinsed with the buffer solution. The resonance frequency was monitored during the injection sequence.

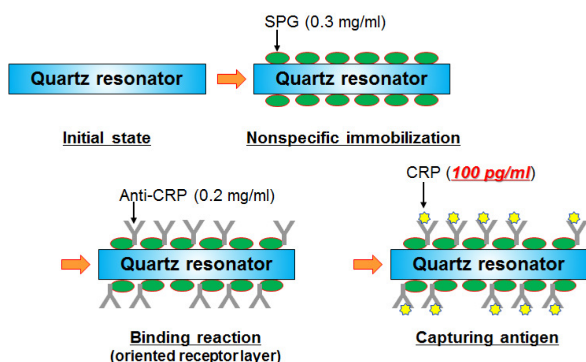

Fig. 2 Schematic of the injection sequence.

3. Results and Discussion

Figure 3 shows an example of the real time monitoring of the assay sequence of the nonspecific adsorption of the SPG molecules, immobilization of oriented anti-CRP receptor layer, binding reaction between anti-CRP and CRP, and dissociation reaction of anti-CRP from CRP. The frequency change due to the nonspecific adsorption of the SPG was 183 Hz (corresponding to 0.12 ng of SPG molecule), and this remains constant until the solution is switched to the buffer solution, indicating the high affinity of the protein and the naked quartz surface even with the nonspecific adsorption. Subsequently, the steep frequency change due to the specific adsorption between SPG and anti-CRP was found to be 764 Hz (corresponding to 0.48 ng of anti-CRP molecule). And the frequency change due to the specific binding of CRP molecules via orientationally immobilized anti-CRP molecules on SPG molecules was then found to be 305 Hz (corresponding to 0.19 ng of CRP molecule). This result indicates that the RAMNE-Q biosensor allows high-sensitive detection to the concentration of the analyte at which the commercial QCMs would have never realized. The frequency change due to the dissociation of CRP molecules by the rinsing with PBS solution flow after binding reaction was not observed.

Figure 4 shows the reaction curves obtained by the injection of CRP solution (100 pg/ml) to the anti-CRP receptor layer adsorbed disorderly and nonspecifically on the quartz surfaces and obtained in **Fig. 3**. The frequency change due to the binding reaction via non-oriented anti-CRP receptor layer increased by 94 Hz after the buffer solution rinsing although the sensor signal is unstable, compared with oriented anti-CRP receptor layer. These show that applying the oriented antibody for the detection of analyte attains high sensitive detection.

We derived thermodynamic parameters from the reaction and dissociation curves between the oriented anti-CRP molecules and CRP molecules as k_a=1.7×10^{9} s^{-1}M^{-1}, k_d=9.5×10^{-3} s^{-1}, and K_A=1.8×10^{11} M^{-1}, (k_a, and k_d denote reaction velocity constants for association and dissociation reactions, respectively, and K_A is the equilibrium constant). This result indicates that the binding reaction via orientated antibody presents high affinity compared with non-orientated antibody[4].

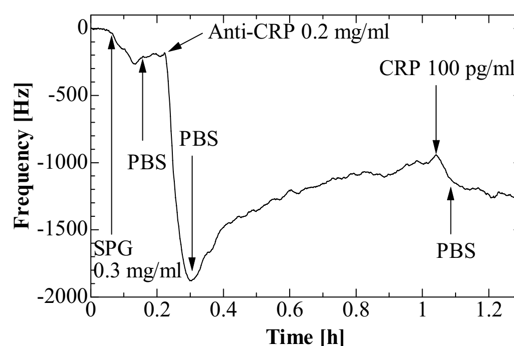

Fig. 3 Behavior of the frequency change caused by the injection of sequence.

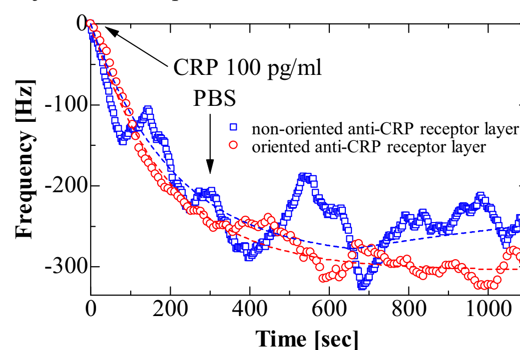

Fig. 4 Binding curves of CRP molecules via non-oriented and oriented anti-CRP molecules.

4. Conclusion

We succeeded in detection of 100 pg/ml CRP via the anti-CRP receptor layer immobilized orientationally and specifically by SPG molecules adsorbed on the quartz crystal nonspecifically. The RAMNE-Q biosensor shows a new possibility beyond the limit of the conventional QCM.

References

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