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Viscoelastic properties of protein layers studied by RAMNE-Q biosensors

高周波 RAMNE-Q バイオセンサーによる蛋白質膜の粘弾性特性の評価

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1. Introduction

The quartz crystal microbalance (QCM) biosensor is a mass-sensitive biosensor. It detects adsorbed mass as the frequency shift of the quartz resonator, when the analyte is adsorbed on the quartz surfaces(1). Therefore, the QCM biosensor can achieve a real-time monitoring of binding reactions. Moreover, it is a label-free biosensor, which allows to evaluate intrinsic interactions among biomolecules and to make the assays more simplified. Thus, the QCM biosensor is a powerful tool for studying interactions among biomolecules.

Recently, QCM biosensors are also used for evaluation of viscoelastic properties of protein layers by measuring dissipation along with the frequency, which is called QCM-D technique (2-3).

Conventional QCM biosensors show lower sensitivity than others that use labels. That issue is due to heavy electrodes attached on the quartz surfaces: The conventional QCM biosensors use AT-cut quartz crystals with resonance frequencies of about 5-10 MHz, corresponding to thickness of 166-330 μm. They have to use Au electrodes to apply effective electric field and make the gold-thiol reaction to immobilize receptor proteins. However, it has been proven that increase of inertia resistance due to those electrodes cause significant decrease of the mass sensitivity(4). The QCM-D biosensor also has some problems about low sensitivity due to low frequencies and low accuracy for measuring dissipation. It needs to measure change in dissipation of quartz resonator, but dissipation is easily affected by many factors, including holding condition of the resonator. These issues make it difficult to evaluate viscoelastic properties of low molecular weight molecules. The best way for evaluating the viscoelastic properties will be using only frequency changes, but this requires extremely high frequency measurements due to less sensitivity of the frequency to the viscoelastic properties than dissipation.

We then developed a resonance acoustic microbalance with naked-embedded quartz (RAMNE-Q), where both surfaces and all sides of an electrodeless naked-quartz resonator are supported without any fixed parts(6-8). In these study, we succeeded in achieving a viscoelastic evaluation with a high-frequency RAMNE-Q biosensor using frequencies over 1 GHz with the fundamental resonance frequency of 58 MHz. This gives us new technique for evaluation of viscoelastic properties that only needs frequency shifts without measuring dissipation.

2. Necessity of high-frequency QCM for viscoelastic properties

For determining viscoelastic properties, we should measure frequency shits of overtones. For example, **Figure 1** shows frequency changes including overtones when a viscoelastic protein layer is adsorbed on the quartz surface for 5 and 55 MHz QCMs. In this calculation, we used the Voight model(9) for a three-layer model, consisting of the quartz-oscillator layer, the viscoelastic protein layer, and the viscous solution layer. The viscoelastic properties of the protein layer causes a discrepancy from the mass-loading effect (Sauerbrey equation) for higher overtones because a very soft layer cannot move on with a high frequency vibration of the substrate, indicating that a higher-frequency QCM can determine the viscoelastic properties only with the frequency measurements.

3. Results and Discussions

We should then take a wider-frequency range measurement than our previous one, where a high-power gated amplifier and superheterodyne spectrometer were adopted.

In this study, we use a network analyzer to achieve higher frequencies for overtones. We used a RAMNE-Q biosensor with 58-MHz fundamental resonance frequency and measured overtones over 19^{th} mode (1.1 GHz) as shown in **Figure 2**. From these measurements, we successfully extracted the viscoelastic properties of receptor proteins and then the adsorbed

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analyte proteins inversely, which will be reported in the symposium.

4. Conclusion

Using RAMNE-Q biosensor with network analyzer, we succeeded in stable measurement for many overtone modes, ranging from fundamental mode (58 MHz) to 19th mode (1.1 GHz). This achievement leads to new technique of high precision and high sensitivity evaluation of viscoelastic properties.

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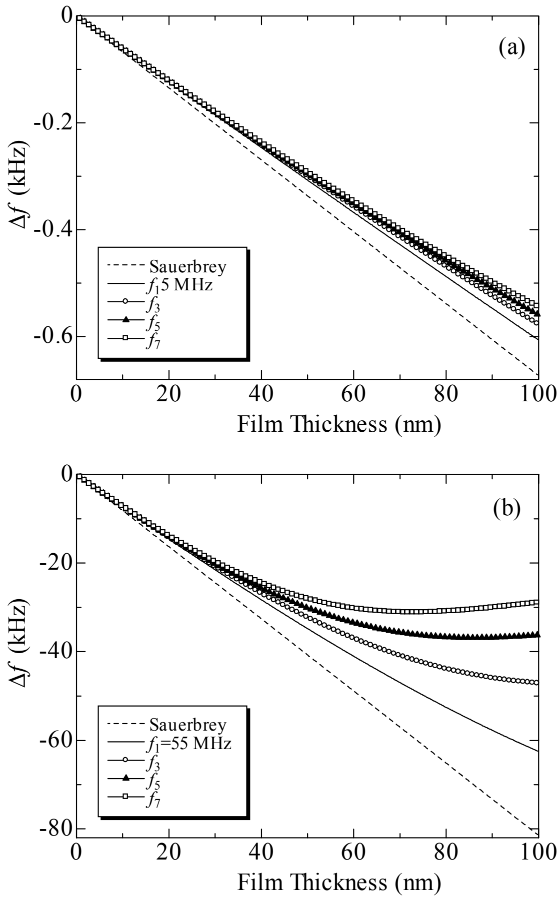

Fig. 1 Theoretical calculation of frequency changes for (a) 5 MHz and (b) 55 MHz QCMs, when a viscoelastic protein layer is adsorbed on the quartz surface. The viscosity and stiffness of the protein layer are 0.01 Ns/m^2 and 100 kPa, respectively.

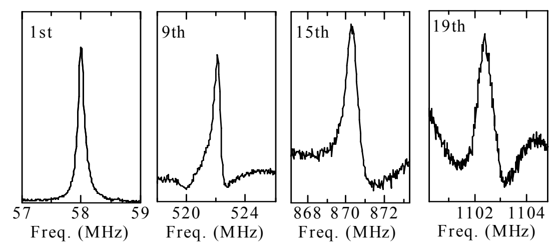

Fig. 2 Resonant spectra of the RAMNE-Q biosensor with fundamental resonance frequency of 58 MHz.