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Mechanism of affinity enhanced protein adsorption on bio-nanocapsules studied by viscoelasticity measurement with wireless QCM biosensor

無線QCM粘弾性計測によるＢＮＣ上の蛋白質補足時の親和性向上機構の解明

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1. Introduction

Bio-nanocapsules (BNC) are known as a useful tool in drug delivery system. Various BNCs have been developed and reported, and we focused on ZZ-BNC, which is BNC with the tandem-form IgG Fc-binding Z domain derived for *Staphylococcus aureus* protein A (Fig. 1) [1]. The ZZ-BNC has a diameter of ~30 nm and molecular mass of 6.57 MDa, and it is capable of capturing about 60 IgG molecules on its surface. It was reported that binding affinity between ZZ-BNC and IgG is higher than that between protein A and IgG by a conventional quartz crystal microbalance (QCM) measurement [1]. The ZZ-BNC is also useful as a sensitizer for the QCM-biosensor measurement, because ZZ-BNC and antibody complexes show very high molecular weight, enhancing the mass sensitivity of QCM [2]. However, mechanism of affinity enhanced IgG adsorption on ZZ-BNC remains unknown.

The QCM biosensor is a mass-sensitive label-free biosensor. It observes interaction between biochemical molecules through change in the resonance frequency of the quartz crystal oscillator. To compare with other biosensors such as surface plasmon resonance (SPR), the conventional QCM biosensor shows a lower sensitivity for targets with smaller molecular weight. One of the causes of the low sensitivity is the presence of gold electrodes and wires attached on the quartz surfaces. To improve the sensitivity, we have developed wireless-electrodeless QCM (WE-QCM) biosensors. The oscillators used in the WE-QCM are electrodeless AT-cut quartz plates, whose thicknesses are significantly smaller than those of conventional QCM (resonance frequencies of our WE-QCM are 54 MHz and 56 MHz).

We deposited Cr and Au ultrathin films (~ 20 nm) on the oscillators to modify the sensor surfaces.

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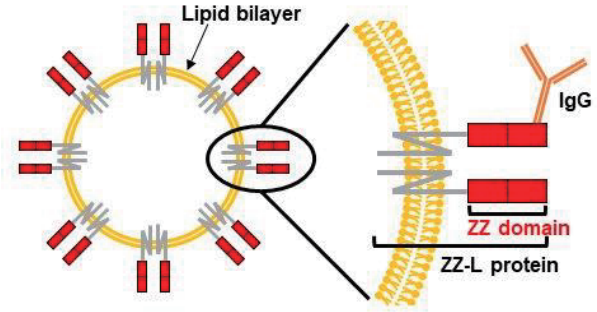

Fig. 1. Illustration of bio-nanocapsule structure of ZZ-BNC.

(Note that the ultrathin Cr and Au films hardly affect the mass sensitivity [3].) Also, the WE-QCM biosensor can measure multiple channels by changing the resonance frequency band of quartz crystal oscillator.

We propose to investigate mechanism of affinity enhanced IgG adsorption on ZZ-BNC by a viscoelasticity measurement using only frequency change. Unlike the existing method known as the QCM-D, our method can determine the viscoelastic properties of protein layers without the less-accurate dissipation measurement. This is made possible because with the high-frequency WE-QCM.

2. Experiment Procedure

We deposited 5 nm Cr and then 15 nm Au thin films on both surfaces of the oscillators. Their surfaces were washed with piranha solution (98% H_2SO_4:33%H_2O_2 = 7:3) for 1 h and rinsed with ultrapure water several times. Their surfaces were cleaned with a UV ozone cleaner for 15 min. The oscillators were then immersed in 10 mM 10-carboxy-1- decanethiol solution overnight at 4°C. After rinsing them with ethanol and ultrapure water, their surfaces were activated by 100 mM EDC

(1-ethyl-3-(3-dimethylaminopropyl) carbodiimide solution for 1 h. After rinsing with ultrapure water, the oscillators were immobilized in 20 μg/ml SPA (Staphylococcal Protein-A) or 20 μg/ml ZZ-BNC solution for 12 h at 4°C for immobilizing them. After rinsing with ultrapure water, they were immobilized in 10 mg/ml BSA (bovine-serum-albumin) solution for blocking remaining activated sites. After rinsing with PBS, we set the oscillators in the multichannel WE-QCM cell. We then injected each concentration human IgG solution with a flow rate of 100 μl/min.

3. Results and Discussion

First, we simultaneously examined human IgG adsorption onto the SPA and ZZ-BNC coated oscillators using WE-QCM repeatedly by injecting 1 μg/ml human IgG into the WE-QCM cell, which was followed by injections of PBS (pH 7.4) and then glycine-HCl (pH 2.2) solutions for dissociating bound human IgG molecules. This cycle was repeated 4 times as shown in Fig, 3(A). The result showed that ZZ-BNC exhibits higher affinity than SPA. This high affinity remained unchanged for repeated use. The same measurement was performed at 0.1 and 1.0 μg/ml human IgG injections, and identical results were obtained (Fig. 3(B)). Furthermore, we measured the binding curves by injecting various concentration of human IgG solutions and determined the equilibrium dissociation (K_D) constants (data not shown). The K_D value between human IgG and SPA is 5.0 ± 3.6 nM and that between human IgG and ZZ-BNC is 1.3 ± 0.6 nM, confirming higher affinity with ZZ-BNC.

Next, we performed simultaneous measurements of overtones up to 9th mode (522 MHz) for adsorption between ZZ-BNC and human IgG. We clearly observed the difference in the frequency change. We think that this difference may have the viscosity effect. Now, we inversely evaluate viscosity using the Voight-Kelvin model under various conditions. We expect that the viscoelasticity measurement is made possible with the WE-QCM biosensor only from frequency information. If this viscoelasticity measurement method by WE-QCM biosensor can be established, it will reveal the mechanism of affinity enhanced protein adsorption on ZZ-BNC.

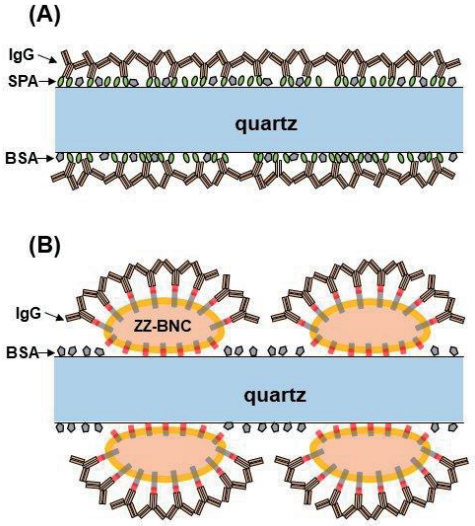

Fig. 2. Schematics of quartz crystal oscillator surfaces on which (A) SPA and (B) ZZ-BNC are immobilized.

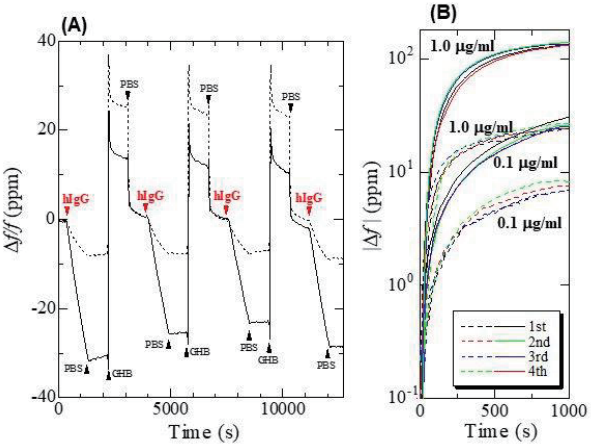

Fig. 3. (A) Frequency responses when 1.0 μg/ml human IgG was injected onto the SPA (dashed line) and ZZ-BNC (solid line) coated quartz crystal oscillators. The cycle of adsorption and removal of human IgG was repeated 4 times. (B) Amount of the frequency changes for 1.0 μg/ml and 0.1 μg/ml human IgG injection onto the SPA (dashed lines) and ZZ-BNC (solid lines) coated quartz crystal oscillator.

References

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