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Evaluations of aptamer-protein interactions using Wireless-electrodeless QCM biosensors

無線無電極QCMバイオセンサを用いたアプタマーとタンパク質間の相互作用評価

Hiroomi Torii[1‡], Masaki Yamato[2], Kentaro Noi[3], Takashi Matsuzaki[2], Hirotsugu Ogi[2], Tetsuo Minamino[2], and Masahiko Hirao[3] ([1] Grad. School of Eng. Sci., Osaka Univ; [2] Grad. School of Med., Osaka Univ; [3] Inst. of Mol. Emb. and Gene., Kumamoto Univ.)
鳥居宏臣[1‡], 山戸昌樹[2], 野井健太郎[3], 松崎高志[2], 荻博次[1], 南野哲男[2], 平尾雅彦[1] ([1]大阪大院 基礎工, [2]大阪大院 医, [3]熊本大 発生研)

1. Introduction

It has been recognized that biosensors are important for two purposes. First, the diagnosis. A specific protein, called a biomarker, reaches higher blood level when the corresponding disease is developed. Detecting the biomarker by biosensors, we can realize the disease. Second, the drug development. In the drug discovery process for searching materials, which specifically bind to causative substance of a disease, the affinity between the candidate and target materials can be evaluated by biosensors.

In the drug development, aptamers attract our attentions significantly as materials alternative to antibodies because of many advantages over antibodies. The aptamer is a single stranded DNA or RNA and takes a unique form through the intermolecular complemental interaction. They can specifically bind to biomolecules like as corresponding antibodies, including targets to which antibodies cannot bind[1]. They can remain the structures at elevated temperatures. The most important advantage is that they are synthesized at low costs. It is possible to identify aptamers which specifically bind to proteins by the established method called SELEX (systematic evolution of ligands by exponential enrichment). However, the specificity of the candidates should be validated, and their K_D values to the target have to be measured.

Aptamers don't have functional groups for labeling. Biosensors, which use labels such as enzyme-linked immunosorbent assay (ELISA), is therefore, inapplicable to evaluate the affinity related with aptamers, and label-free biosensors are necessary to investigate interactions between aptamaers and target proteins. However, strong negative charge of aptamers make many biosensors unavailable. Then, we apply the wireless-electrodeless QCM (WE-QCM) to evaluate aptamer-protein interactions.

QCM is a mass-sensitive biosensor. The resonant frequency of the quartz resonator shifts depending on the adsorbed mass on the surface. Measuring the frequency change in real time, we can detect the target proteins in the solution and evaluate the affinity between the immobilized proteins on the surface and surrounding target proteins in the solution. A conventional QCM has gold-electrode layers on both surfaces. To supersensitize the QCM, we need to thin the quartz resonator because it is a mass detector. (Gold is denser than quartz.) Even if we reduce the weight of the quartz resonator by thinning it, the inertia resistance caused by the gold electrodes get to have a large effect on the resonance frequency of the quartz resonator, giving a sensitivity limit. We then removed the gold electrodes and developed WE-QCM[2].

We thinned the quartz crystals by a factor of ~0.01 compared with conventional oscillators and supersensitized the QCM. Additionally, there are many improvements; it can be used both sides of the sensor and can be used repeatedly. Moreover, we established the multichannel WE-QCM system. An external electric field is contactlessly applied to the oscillator. The surface polarization switches quickly and the quartz resonator does not show effective charge seemingly, being applicable to bioassays with aptamers. In this study, we apply the WE-QCM to study aptamer-protein interactions for the first time. Heparin-binding EGF-like growth factor (HB-EGF) is highly expressed on different cancer cells and has been studied for target protein of drug delivering system (DDS)[3]. We apply the HB-EGF as a model target to evaluate the availability of the WE-QCM to the aptamer-related bioassay.

2. Experimental procedure

We used two-channel WE-QCM system with resonance frequencies of 58 and 60 MHz. The surfaces of the two quartz resonators were coated with 20-nm thick gold thin films. We used aptamers consisting of 116 mer. Before use, they were heated at 95 ºC for 3 min in the incubator, and

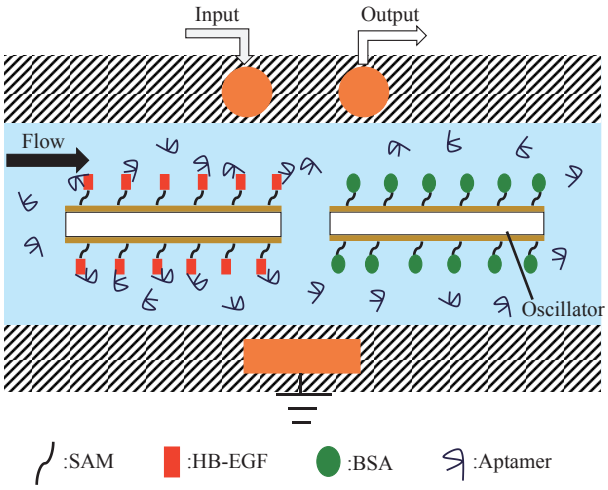

Fig. 1. Experimental schematic of evaluating aptamer-proteins interaction by WE-QCM.

cooled rapidly in ice. Then, they were heated at 37 °C for 15 min in the incubator, and diluted given concentration with the buffer.

Before immobilization, the two sensor chips were cleaned by a piranha solution (H_2O_2:H_2SO_4 = 3:7), rinsed with ultrapure water, and exposed to ultraviolet light. Then, they were incubated in 10 mM 10-carboxy-1-decanethiol overnight to form the self-assembled monolayers on their both surfaces. After rinse with ultrapure water, they were immersed in 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) for 1 h. and then rinsed with ultrapure water.

One chip was immersed in 10 μg/ml HB-EGF solution for the positive channel for 10 h to immobilize the target proteins, and it was in a 10 mg/ml bovine serum albumin (BSA) solution for 1 h to block the remaining active sites. The other chip was immersed in the 10 mg/ml BSA solution for 10 h as the reference channel.

We monitored the two resonance frequencies at the same time and with the same conditions by our own multichannel measurement system as illustrated in Fig. 1. The vibrations of the oscillators are excited and received by two line antennas located at the upper part of the flow channel contactlessly. When aptamers are flowed, they interact with the proteins and are captured on the positive channel, leading to the decrease of the resonance frequency.

We prepared three aptamer solutions with concentrations of 1, 5, and 20 nM, and we injected them sequentially. At first, we flowed the running buffer till resonance frequencies of the oscillators were stable. Then we flowed the aptamer solutions.

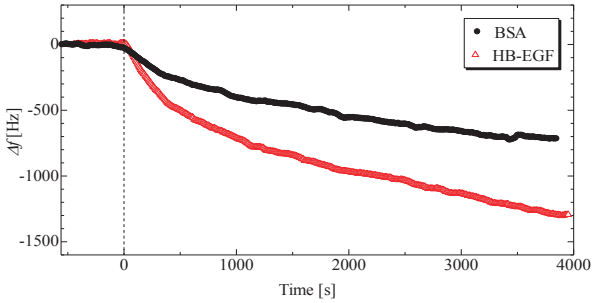

Fig. 2 Comparison plots about frequency changes by interactions of aptamer-HB-EGF and aptamer-BSA. The concentration of the aptamer was 20 nM.

3. Results and discussions

Figure 2 shows an example of the result for an aptamer. The difference between the HB-EGF immobilized positive channel and BSA immobilized negative channel is clear; this aptamer binds more specifically to HB-EGF than BSA. We detected aptamers with a concentration of 1 nM, indicating that our WE-QCM can measure the aptamer-protein interaction even at such a low concentration because of the supersensitized WE-QCM system. Note that we failed to detect the binding reaction with a surface-plasmon-resonance biosensor even with much higher concentration solution of 80 nM. Moreover, we can determine the K_D value for the interaction with the different-concentration measurements. In the case of the binding system in Fig. 2, the K_D value is of the order of 10 nM, being comparable with that between the antigen-antibody interaction. Thus, the WE-QCM can be used to find an aptamer to attach to the target protein specifically.

4. Conclusion

In this study, we successfully evaluated aptamer-protein interactions by the multichannel WE-QCM system. It detected the binding reactions at a low-concentration aptamer solution and provided the binding affinity. In synthesizing aptamers, the shorter the sequence is, the lower the cost becomes. By evaluating interactions between shorter aptamers and proteins, the WE-QCM will contribute to diagnosis and drug development. Considering the difficulty of other biosensors to evaluate aptamer-related interactions, the WE-QCM will play a crucial role in the SELEX procedure.

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