2P3-11

Analysis of reaction rate in chemical reaction between biotinylated bubbles and streptavidin

ビオチン修飾バブルとストレプトアビジン間の化学反応における反応速度の解析

Yuta Otsuki[1†], Kenji Yoshida[2], Yasuhiro Yokoi[1], Yoshiaki Watanabe[1]
([1]Doshisha Univ.; [2]Chiba Univ.)
大月　裕太[1†]　吉田　憲司[2]　横井　康弘[1]　渡辺　好章[1] ([1]同志社大学　[2]千葉大学)

1. Introduction

Targeted microbubbles (TMBs) can be applied to ultrasonic molecular imaging, which detects echo signals from bubbles specifically absorbed to the target such as lesion and visualizes the size, shape and state of the tissue. To improve the quantitative performance as molecular imaging, a previous *in-vivo* study proposed a method to distinguish bubbles bound to target tissue from freely floating bubbles using a time-intensity curve of echo signal [1].

To explain the time-intensity curve of echo signal, it is important to understand the affinity between TMBs and target molecule from the viewpoint of chemical kinetics. In the previous study, we constructed an evaluation system using a quartz crystal microbalance (QCM) sensor and demonstrated that the specific adsorption of TMBs to target molecules could be evaluated by analyzing the change of the resonance characteristics of the QCM[2,3]. This report discusses the time constant in chemical reaction between biothinylated bubbles as TMBs and streptavidin as target molecule based on the experiments using a flow system evaluation system.

2. Experimental system

A QCM configured with electrodes on both side of AT-cut quartz (QA-A5M-AU (M) (SEP), Seiko EG&G) was employed as sensor. Its fundamental resonance was 5 MHz. The diameter and area of electrode were 5 mm and 20 mm^2, respectively. The frequency characteristic of the admittance around the third resonance was measured from the viewpoint of electrical impedance matching.

Fig. 1 shows a flow system composed of syringe pump, injector valve, flow cell, network analyzer (E5071B, Agilent Technologies, USA) and PC for control. The QCM was fully built into the flow cell. In the experiment, streptavidin as target was sent and fixed on the QCM surface via a preliminarily-fixed self-assembled monolayer. Then, the suspension of biotinylated bubbles was sent to the surface of QCM using the syringe pump. During the above process, reflection coefficient S_{11} was measured every 6 sec by using a network analyzer and converted into admittance. The resonance frequency and Q value were calculated and recorded in real time.

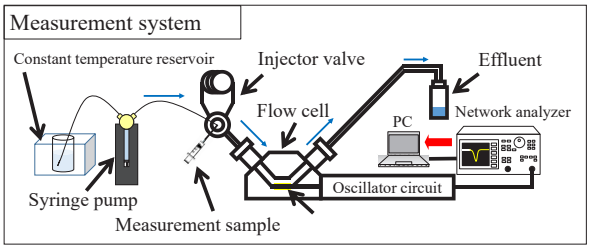

Fig.1 Flow system

3. Evaluation of the affinity between TMBs and target molecules

Fig. 2 shows the time-Δf curve during the reaction between biotinylated bubble and streptavidin, where Δf means the shift of resonant frequency of QCM. The concentrations of bubbles in suspension were 1440 bubbles/mm^3. The resonance frequency decreased exponentially because the TMBs starts to adsorb to streptavidin on the surface of the QCM. When the resonant frequency seemed to be saturated, ultrapure water (UPW) was sent to remove nonspecifically-adsorbed bubbles. Fig. 2 shows the nonspecifically-adsorbed bubbles have been removed because the resonance frequency slightly increases in this washing process. f_{0_a} was expressed as the amount of frequency shift due to adsorption bubbles (specifically adsorbed bubbles and nonspecifically-adsorbed bubbles).

4. Effect of non-adsorbed bubbles

Time constant in reaction between biotinylated bubble and streptavidin could not be evaluated from the result as shown in Fig. 2 because it included the effect of freely floating bubble (non-absorbed bubbles). To solve this problem, we model the temporal change of the resonance frequency,

$$\Delta f = f_{0\_a} * \left(1 - \exp\left(-\frac{t}{\tau_{0_a}}\right)\right) + f_{0\_na} * \left(1 - \exp\left(-\frac{t}{\tau_{0_{na}}}\right)\right) \quad (1)$$

The first and second terms represent frequency shift due to adsorbed bubbles and non-adsorption bubbles, respectively.

To obtain time constant τ_{0_a} by fitting the curve in Fig. 2, we preliminary need to know the coefficient of f_{0_a}, f_{0_na} and τ_{0_na}. f_{0_a} could be obtained from Fig.2.

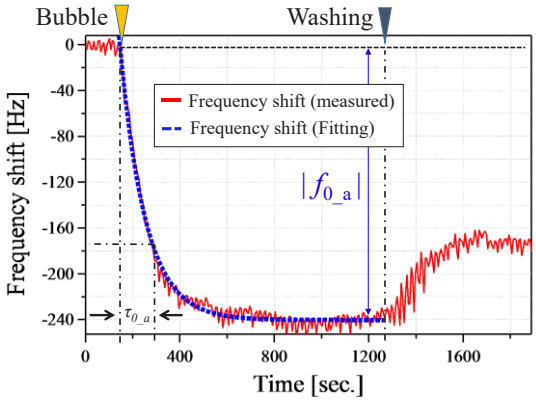

Fig.2 Frequency shift caused by biotinylated bubbles

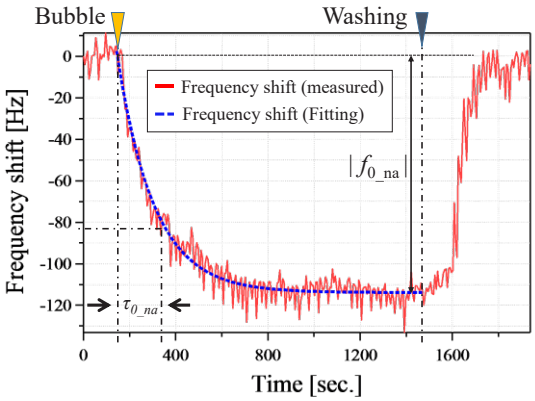

Fig.3 Frequency shift by control bubbles (non-biotinylated bubble)

It was assumed that almost all control bubble (not modified with biotin) cannot be adsorbed to QCM, i.e. f_0 and τ_0 for control bubble are approximately equal to f_{0_na} and τ_{0_na} in Eq.(1). Under this assumption, the coefficients f_{0_na} and τ_{0_na} were estimated from experiments using control bubbles. We prepared bubble suspensions with four different number density and evaluated f_{0_na} and τ_{0_na} by fitting time-Δf curve with exponential function,

$$\Delta f = f_{0\_na} * \left(1 - \exp\left(-\frac{t}{\tau_{0\_na}}\right)\right) \qquad (2)$$

Fig. 3 shows time-Δf curve for control bubble. The resonance frequency exponentially decreases and return to the initial value after washing process. The results suggested that no specific adsorption of the control bubbles occurred in this case. It was investigated how f_{0_na} and τ_{0_na} depend on number density of control bubbles. We confirmed that τ_{0_na} decreased with increasing in number density of bubbles (see Fig. 3), although there were no trend for f_{0_na}.

τ_{0_a} for the adsorption bubbles was estimated by substitute f_{0_a}, f_{0_na} and τ_{0_na} into eq.(1) and fitting. Because f_{0_na} has no dependence on number density of bubble, we used its average value of - 118.3 Hz. In addition, f_{0_na} was converted by considering the ratio of amount of f_{0_a} and f_{0_na} in the measurement of biotinylated bubbles. **Fig. 4** shows the dependence of the time constants τ_{0_na} and τ_{0_a} on the number density of bubbles. The value of the coefficients A and B was obtained by fitting the relationship between the time constants τ_{0_na} or τ_{0_a} and the number density of bubbles with $\tau = 10^B * C^A$ (C is the number density of bubbles). The results are shown in **Table 1**. The time constant decreased with increasing in the number density of bubbles. It was found that τ_{0_a} was always smaller than τ_{0_na}.

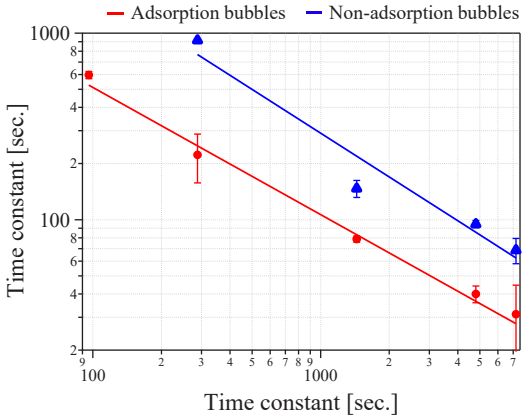

Fig.4 Time constant VS Number of bubbles in suspension

Table1 Measurement of time constant.

	τ_{0_na} (sec.)	τ_{0_a} (sec.)
A	**-0.78**	**-0.68**
B	**4.8**	**4.07**
R^2	**-0.939**	**0.940**

5. Summary

This report demonstrated that chemical reaction between biotinylated bubbles and streptavidin can be evaluated by using a flow system composed of QCM sensor. In particular, we analyze the time constant in reaction and quantitative relationship between the time constant and the number density of bubbles. Because we used several assumptions for analyzing, we will verify whether they are correct or not by incorporating microscope into the proposed system.

Acknowledgment

This work was partially supported by JSPS KAKENHI Grant No. 13303779, The Murata Science Foundation, and the Ono Charitable Trust for Acoustics.

References

1. James shue-min yeh, et al. Ultrasound in Med. & Biol., Vol. 41, No. 9, pp. 2478–2496, 2015.
2. T. Muramoto, et al, Ultrasound Med.Biol. 40, 2014.
3. R. Shimoya, et al. Japanese Journal of Applied Physics 54, 2015.