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Validation of model of kinetics between streptavidin and biotinylated microbubbles.

アビジンとビオチン化マイクロバブル間の反応速度論のモデル化と妥当性の検証

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1. Introduction

Recently, microbubbles (MBs) which can bind to specific molecules are expected to be applied as contrast agents for molecular ultrasonic imaging. In previous studies, targeted MBs adsorbing to VEGFR-2, $a_v\beta_3$, and Pselectin have been developed and tested in animal model experiment. It has been pointed that the binding force depend on the ligand-receptor type and the surrounding circumstance such as shear force. To realize effectiveness of ultrasound molecular imaging, it is important to deeply understand the kinetics between MBs and target molecules. In previous researches, we have suggested the sensing system based on a quartz crystal microbalance (QCM) for monitoring the reaction between MBs and target molecule. It was found that the density of MBs on the QCM surface are correlated with the change in resonant frequency of QCM i.e. frequency shift Δf. This implied that the kinetics between MBs and target can be analyzed based on the Δf-time curve. This report models the reaction kinetics between MBs and target, and analyzes the effective association and dissociation rate constants under the interaction between biotinylated MBs (BMBs) and streptavidin (SA).

2. Modeling the reaction kinetics

The chemical equilibrium of analyte A and target B is expressed by the following formula.

$$[AB] \rightleftarrows [A] + [B] \qquad (1)$$

Analyte A, target B, and reactant correspond to biotin, streptavidin, and biotin binding to streptavidin, respectively. The concentration (C_A) of analyte A and the concentration (C_{AB}) of reaction product AB can be

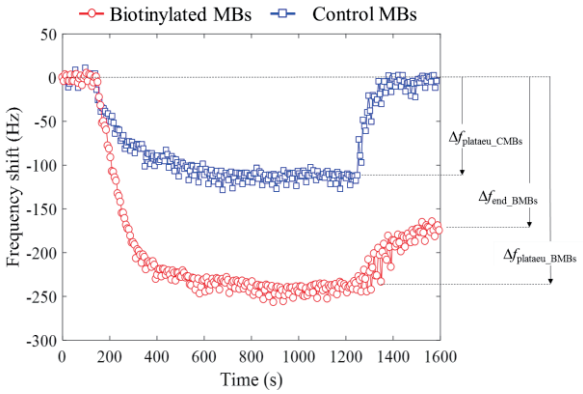

Fig. 1 Δf-time curve in case of biotinylatedMBs

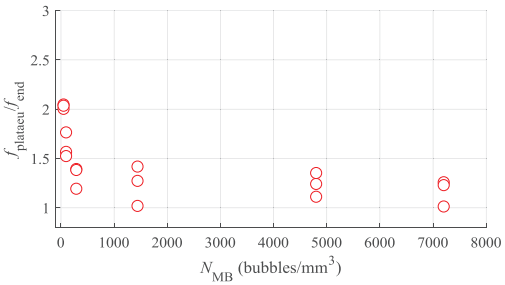

Fig 2. $\Delta f_{plateau}$ /Δf_{end} -N_{MB} characteristic in cases of biotinylated MBs

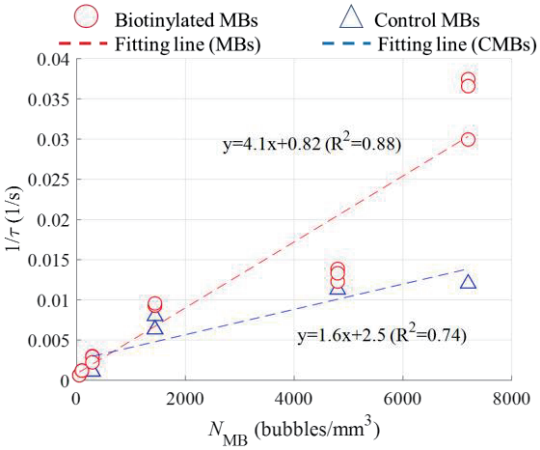

Fig 3. 1/τ-NMB characteristic in cases of biotinylated MBs and control MBs, where dashed lines show fitting line in each case.

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replaced by the number density of BMBs (N_{MB}) and that of MBs bound to SA (N_{MB_SA}), assuming of the proportional relationship such as $C_A = \alpha N_{MB}$ and $C_{AB} = \alpha N_{MB\_SA}$ where α is constant. When the suspension of BMBs with constant number density of N_{MB} keeps flowing to QCM, the differential equation for N_{MB_SA} can be written as

$$\frac{dN_{MB\_SA}}{dt} = k_a N_{MB} C_{SA0} - (k_d + \alpha k_a N_{MB}) N_{MB\_SA} \quad (2)$$

where k_a and k_d are association and dissociation constant. C_{SA0} is defined as the initial concentration of streptavidin on QCM. Assuming that Δf is proportional to N_{MB_SA} ($=\beta\Delta f$) yields the differential equation for Δf,

$$\frac{d\Delta f}{dt} = \frac{k_a N_{MB} C_{SA0}}{\beta} - (k_d + \alpha k_a N_{MB})\Delta f. \quad (3)$$

The solution of Eq.(2) can be written as

$$\Delta f = \lambda e^{-t/\tau} + \Delta f_{plateau}, \quad (4)$$

By substituting Eq.(3) to Eq.(2), the following equations can be obtained,

$$\frac{1}{\tau} = k_{ea} N_{MB} + k_d \quad (5)$$

where k_{ea} is defined as the effective association constant ($=\alpha k_a$). This equation suggest that we can quantify the effective association constant k_{ea} and dissociation constant k_d by measuring the time constant τ in some conditions for the number density NMB of BMB.

The non-specifically binding between BMBs and SA should occur in the reaction. To analyze the association rate of specifically bound MBs separately with non-specifically bound MBs in the case of BMBs, we introduce the following relationships

$$\begin{cases} k_{ea} N_{MB} = k_{ea\_s} N_{MB\_s} + k_{ea\_ns} N_{MB\_ns} \\ N_{MB} = N_{MB\_s} + N_{MB\_ns} \end{cases} \quad (6)$$

where the subscript 's' and 'ns' indicate the parameters of specifically- and non-specifically bound MBs, respectively. Assuming that the rate constants of the non-specifically bound MBs could be replaced by those of CMBs and the ratio N_{MB}/N_{MB_s} is equal to $\Delta f_{plateau}/\Delta f_{end}$. Δf_{end} means the frequency shift due to the BMBs and can be quantified by measuring the Δf after washing unbounded MBs. Thus, the association rate of the specifically-bound MBs can be written as

$$k_{ea\_s} = \frac{\Delta f_{plateau}}{\Delta f_{end}}(k_{ea} - k_{ea\_ns}) + k_{ea\_ns}. \quad (7)$$

Table 1. Effective association and dissociation rate constant and dissociation constant

	k_d ($\times 10^{-3}$ 1/s)	k_{ea} ($\times 10^{-6}$ mm^3/bubbles/s)	k_{ea_s} ($\times 10^{-6}$ mm^3/bubbles/s)
Control MBs	2.5 ± 1.1	1.6 ± 0.34	-
Biotinylated MBs	0.82 ± 1.3	4.1 ± 0.36	4.85

3. Results

By using a QCM interpolated to a flow system[1], the resonant frequency shift (Δf) under the interaction between MBs and SA was monitored in real-time. **Figure 1** shows the typical Δf-time curve in case of BMB and CMBs. The ultrapure water was flowed for washing non-specifically bounded MBs at the end step. Detailed experimental conditions can be referred in Ref 1.

Figure 2 shows that $1/\tau$-N_{MB} characteristic. In both cases of BMBs and CMBs, $1/\tau$ seemed to increase with increasing in N_{MB}. The slope of the curve in case of BMBs was larger than that in case of CMBs. This results suggested that the QCM system could characterize the difference in kinetics between BMBs and CMBs. **Figure 3** show the $\Delta f_{plateau}/\Delta f_{end}$ in various conditions for N_{MB}. It was found that there were few dependences of $\Delta f_{plateau}/\Delta f_{end}$ on N_{MB}. We quantified the association rate of the specifically-bound BMBs k_{ea_s} by using Eq. (7) assuming that $\Delta f_{plateau}/\Delta f_{end} = 1.3$. The resultant parameters k_e, k_{ea} and k_{ea_s} are summarized in **Table 1**.

4. Summary

We modeled the kinetics in the interaction between TMBs and target molecule using the theory for chemical kinetics. The results clearly showed that the QCM system could characterize the difference in performance between targeted- and non-targeted MBs although the accuracy of our method should be validated by compared with other method.

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References

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