3Pa3-6

Proceedings of Symposium on Ultrasonic Electronics, Vol. 32 (2011) pp. 361-362
8-10 November, 2011

Molecular-Mass Analysis of Proteins in Liquids Using a High Frequency Wireless-Electrodeless Quartz Crystal Microbalance Sensor

高周波無線・無電極水晶振動子センサによる液中タンパク質の分子量測定法の開発

Taiji Yanagida[1†], Hirotsugu Ogi [1], Masayoshi Nishiyama[2] and Masahiko Hirao[1] ([1] Graduate School of Engineering Science, Osaka Univ.; [2] Renovation Center of Instruments for Science Education and Technology, Osaka Univ.)

柳田泰次 [1‡], 荻博次 [1], 西山雅祥 [2], 平尾雅彦 [1], ([1] 大阪大大学院基礎工研究科, [2] 大阪大科学教育機器リノベーションセンター)

1. Introduction

The piezoelectric quartz crystal microbalance (QCM) is a mass-detectable sensor, which can detect mass change on the surface of the quartz oscillator through the change in the resonance frequency of the oscillator. Initially, the QCM was used for dry measurements such as a film-thickness meter. Recently it is possible to carry out measurements under liquid conditions, allowing us to use QCM as biosensors for detecting biomolecular interactions. Here, we propose a novel application of QCM as a molecular mass sensor of proteins in liquids.

In the use of QCM in liquids, density and viscosity of the liquid affect the resonance frequency. Thus, observed frequency change (Δf) is represented as a sum of mass effect and viscosity effect[1, 2],

$$\Delta f = -\frac{2\Delta m}{A\sqrt{\rho_q \mu_q}} f_0^{\,2} - \sqrt{\frac{\rho_l \eta_l}{\pi \rho_q \mu_q}} f_0^{\frac{3}{2}} \qquad (1)$$

where Δm denotes the absorbed mass, A is the sensor surface area, f_0 is the fundamental resonance frequency of the sensor, ρ_q and μ_q are density and shear modulus of quartz, and ρ_l and η_l are density and viscosity of the surrounding liquid. In Eq. (1), to detect biomolecular interactions more quantitatively and sensitively, higher fundamental resonance frequency QCM is required.

The fundamental resonance frequency of QCM can be improved by thinning the quartz crystal. We then proposed a wireless- electrodeless (WE) technique and achieved advanced QCM biosensors using much thinner quartz oscillators than those used in conventional QCMs by a factor of 0.03, showing fundamental frequencies up to 180 MHz[3-6]. Using this technique will allow highly quantitative and sensitive detection of biomolecular interactions in liquids to determine the molecular mass of proteins.

Email:ogi@me.es.osaka-u.ac.jp

In this study, we propose a molecular mass analysis using the quantitative high frequency WE-QCM, and analyze the molecular mass of staphylococcal protein A (SPA) via immunoglobulin G (hIgG) immobilized on the quartz crystal surface nonspecifically[5, 6].

2. Principle

The reaction between analyte A and receptor B is represented as

$$\mathrm{A} + \mathrm{B} \underset{k_d}{\overset{k_a}{\rightleftarrows}} \mathrm{AB} \qquad (2)$$

where k_a and k_d are association-velocity constant and dissociation-velocity constant, respectively. This equilibrated reaction proceeds exponentially,

$$[\mathrm{AB}] = [\mathrm{AB}]_e \{1 - \exp\{-(k_a[\mathrm{A}]_0 + k_d)t\}\} \qquad (3)$$

where t denotes time, [X] indicates the concentration of X, and $[\mathrm{A}]_0$ and $[\mathrm{AB}]_e$ are initial concentration of A and equilibrium concentration of AB. **Fig. 1** shows the frequency responses of QCM for this reaction, where t_i denotes the injection time of B, $\alpha_1 = k_a[\mathrm{A}]_0 + k_d$, and $\alpha_2 = k_d$. The concentrations $[\mathrm{B}]_0$ and $[\mathrm{AB}]_i$ ($t = t_i$) are calculated by the frequency changes Δf_A and Δf_B,

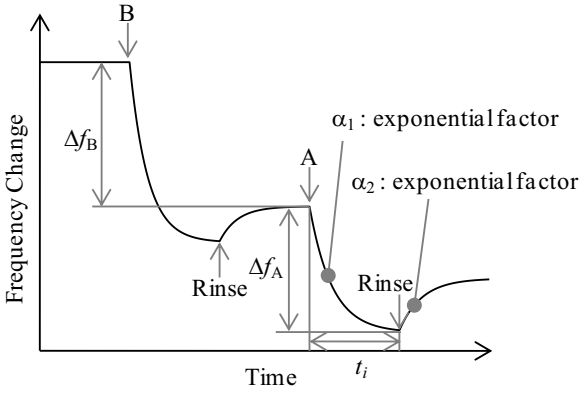

Fig. 1 Frequency changes of QCM during molecular mass analysis.

$$[\mathrm{B}]_0 = \frac{\Delta f_\mathrm{B}}{f_0}\frac{M_q}{m_\mathrm{B}A}$$
$$[AB]_\mathrm{i} = \frac{\Delta f_\mathrm{A}}{f_0}\frac{M_q}{m_\mathrm{A}A}\left\{1-\exp\left(-\alpha_1 t_\mathrm{i}\right)\right\} \quad (4)$$

where m_A and m_B are the molecular mass of A and B, and M_q is the mass of the oscillator. Then m_A is represented as

$$m_\mathrm{A} = \left(1-\frac{\alpha_2}{\alpha_1}\right)^{-1}\frac{\Delta f_\mathrm{A}}{\Delta f_\mathrm{B}} m_\mathrm{B}\left\{1-e^{-\alpha_1 t_\mathrm{i}}\right\}^{-1} \quad (5)$$

Using this convenient principle, the unknown molecular mass of the protein A in the liquid is determined by simple flow experiment of QCM by capturing it with protein B, whose molecular mass is known.

3. Experiment

The 9.1-9.4-μm thick AT-cut bare quartz plates, whose fundamental resonance frequencies were 175-181 MHz, were used. The crystals were cleaned in a piranha solution (98% H_2SO_4 : 33% H_2O_2 = 7 : 3) for 10 min. After rinsing with ultrapure water, the sensor chips were set into the flow injection system for continuous and stable monitoring of the resonance frequency. The temperature in the cell was maintained at 25 ± 0.1ºC. The carrier solution was phosphate buffered saline (PBS, pH 7.4) solution, and the flow rate was 100 μl/min. After the resonance frequencies were stable, the hIgG solution containing 0.25 mg/ml was injected for 50 min to immobilize them on the sensor surfaces nonspecifically, which was followed by the rinsing procedure with the PBS flow. Then the SPA solution (1 μg/ml) was injected for 50 min, which was followed by rinsing procedure.

4. Results and Discussion

Fig. 2 shows the frequency responses through the injection sequence. The large frequency changes by the injection of the 0.25 mg/ml hIgG/PBS solution indicate that 684 ± 87 fmol hIgG were immobilized on the sensor surfce nonspecifically. Then, the injection of the 1 μg/ml SPA/PBS causes the exponential frequency decreases by SPA-IgG binding. Following that, the PBS rinsing causes the exponential frequency increases by SPA dissociation. Using these response curves, the molecular mass of SPA was calculated by Eq. (5) (α_1 and α_2 were obtained by fitting association and dissociation curves using the least square method). As a result, SPA molecular weight was determined

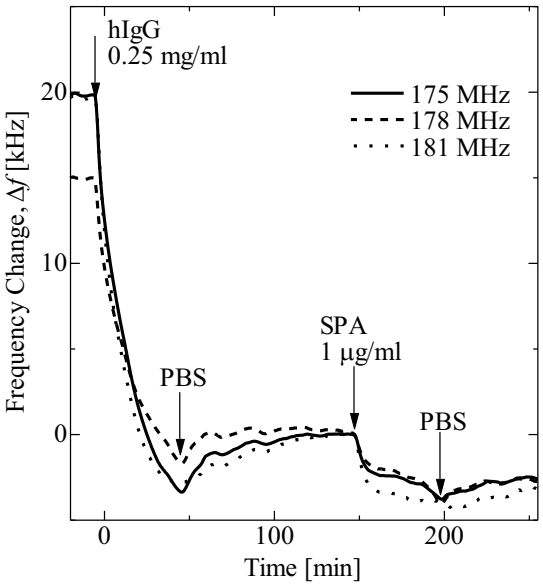

Fig. 2 Frequency change behaviors for injection of 0.25 mg/ml hIgG solution and 1 μg/ml SPA solution.

to be 36.1 ± 6.2 kDa, which is comparable to true value (45 kDa).

We achieved the molecular mass determination of SPA with the simple principle and experiment. Using the high frequency WE-QCM, low molecular mass proteins can be analyzed because it's not only quantitative but also sensitive. Moreover, total measurement time in this experiment was 4 h, which may be shortened by increasing the flow rate, for example.

5. Conclusion

We proposed a novel molecular mass analysis of proteins in liquids using the high-frequency WE-QCM with a convenient principle. Using the method, we analyzed molecular mass of SPA by SPA-IgG binding system.

References

1. G. Sauerbrey: Z.Phys. **155** (1959) 206.
2. K. K. Kanazawa, J. G. Gordon II, Analytica Chimica Acta, **175**, (1985) 99.
3. H.Ogi, K. Motohisa, K. Hatanaka, T. Ohmori, M. Hirao, and M. Nishiyama, Jpn. J. Appl. Phys. **46** (2007) 4693.
4. H. Ogi, Y. Fukunishi, T. Omori, K. Hatanaka, M. Hirao, and M. Nishiyama, Anal. Chem. **80** (2008) 5494
5. H. Ogi, Y. Fukunishi, H.Nagai, K. Okamoto, M. Hirao, and M. Nishiyama, Biosens. Bioelectron. **24** (2009) 3148..
6. H. Ogi, H. Nagai, Y. Fukunishi, T. Yanagida, M. Hirao, M. Nishiyama, Jpn. J. Appl. Phys. **49** (2010) 07HD07.