Measurement of Reaction Rate between DNA and Polyethylenimine using Quartz Crystal Microbalance and Surface Plasmon Resonance

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Polyethylenimine (PEI) is one of the most effective polymers for non-viral gene delivery; however, the mechanism by which DNA binds to PEI remains unclear. In this study, the reaction rate between DNA and PEI was determined using quartz crystal microbalance (QCM) and surface plasmon resonance (SPR). The association rate constant was determined as 0.8–1 s$^{-1}$ mM$^{-1}$, and the dissociation rate constant was found to be 1–2 ×10$^{-3}$ s$^{-1}$. The data obtained from these measurements could be explained using the two-site fitting model. One proposed binding site for the DNA was the PEI; however, the DNA could also have bound directly to the gold surfaces of the sensor devices. Furthermore, the suitability of this two-site model for DNA binding was evaluated in this study.

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I. INTRODUCTION

Gene therapy is a potentially promising strategy for treating hereditary diseases [1]. Although the majority of gene delivery systems are based on viral carriers, these have certain shortcomings that have limited their wider applications. Non-viral gene delivery systems have attracted considerable attention as they have the potential to overcome these limitations [2]. Polyethylenimine (PEI) is one of the most effective polymers for non-viral gene delivery [3-5]. The efficient transfection of DNA by PEI is attributed to its property of condensing DNA molecules into nanoparticles. Although several reports examined the details of DNA-PEI interaction, the underlying mechanism remains unclear.

Quartz crystal microbalance (QCM) is a remarkable method that facilitates the kinetic assessment of biomolecular interactions without labeling [6]. A host molecule is immobilized on the quartz oscillator surface and the analyte is injected. When the analyte molecule is captured by the host molecule, the mass of the oscillator increases, resulting in a decrease in the frequency of oscillation. The change in oscillation frequency is proportional to mass adsorbed to the sensor surface, enabling quantitative determination of adsorption.

Another excellent label-free method for the kinetic assessment of bio-molecular interactions is surface plasmon resonance (SPR) [7]. The host molecule is immobilized on a gold surface, which is irradiated through the back surface by a light beam, and the analyte is then injected. The binding reaction between the host and analyte molecules at the gold surface alters the coupling condition between the evanescent field generated by the incident light and the surface plasmon wave. The incident angle of the light is thus changed during the binding reaction and this change measured.

Here, we applied both QCM and SPR to determine the reaction rate between DNA and PEI. The association rate constant for binding was found to be 0.8–1 s$^{-1}$ mM$^{-1}$, and the dissociation rate constant 1–2 ×10$^{-3}$ s$^{-1}$. We hypothesized that a two-site fitting model, which involves the binding of DNA to two sites in the QCM and SPR sensors, could explain the data obtained from these measurements. In addition to binding to the PEI, the DNA could have possibly bound to the gold surfaces of the QCM or SPR sensors. To our knowledge, this is the first report to determine the rate and mechanism underlying the binding reaction between DNA and PEI.

II. EXPERIMENTAL

DNA was extracted from salmon milt with 1% sodium dodecyl sulfate and chloroform. The quality of DNA was confirmed by spectrophotometry: A 260 nm: 280 nm absorbance ratio of 1.8 indicated high DNA purity. The DNA and 25 kDa branched PEI (Sigma, Japan) were dissolved in 10 mM HEPES buffer (pH 6.8). An AFFINITY QN$\mu$ (Initium, Japan) QCM instrument was used, with a 27-MHz QCM plate and a RANA Personal SPR Sensor (Kyushu Keisokki, Japan) was used for the SPR analyses. The gold electrode was cleaned with 1% SDS and piranha solution (3 parts H$_2$SO$_4$ : 1 part 30% H$_2$O$_2$) before PEI immobilization. Miyamoto et al. have reported that polyamines can be immobilized on gold surfaces via multipoint coordination of their tertiary amino groups [8]. PEI was immobilized on the gold surface by contact with a 0.2 M (monomer residue molar concentration) PEI solution for 15 minutes. The amount of immobilized PEI on the sensing area was 5.5 ± 0.5 ng/mm$^2$ for QCM and 5.6 ± 3.7 ng/mm$^2$ for SPR. Measurements were made following injection of the DNA solution (10 mM HEPES, pH

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III. RESULTS AND DISCUSSIONS

Figure 1 illustrates the time courses of the frequency change during QCM and the resonance signal change of SPR in response to addition of the DNA solution. The time taken to reach a constant frequency or signal increased in a DNA-concentration dependent manner.

Figure 2 shows the frequency changes of QCM in response to addition of 0.24 mM DNA solution into a cell containing immobilized PEI. When a single site model was used (Eq. (1)), the frequency change observed was proportional to the quantity of DNA bound to PEI ($\Delta m$ of Eq. (2)). In Eq. (2), $k_{\text{obs}}$ is an observed association rate constant which is expressed as a function of total DNA concentration (Eq. (3)). In Eq. (3), $k_1$ is an association rate constant and $k_{-1}$ is a dissociation rate constant.

$$ DNA + PEI \xrightarrow{k_{\text{obs}}^{\text{single}}} DNA-PEI \quad (1) $$

$$ \Delta m_t = \Delta m_{\text{max}} \{1 - \exp(-k_{\text{obs}} t)\} \quad (2) $$

$$ k_{\text{obs}} = k_1 [\text{DNA}] + k_{-1} \quad (3) $$

As the single site model corresponded poorly with the experimental data (dashed-dotted line in Fig. 2), we postulated the existence of a second site that the DNA was bound to ($H_{\text{site2}}$ in Eq. (4)). The frequency change for the two-site model is the sum of the interaction with PEI and with $H_{\text{site2}}$ (Eq. (5)). The two-site model corresponded well with the experimental data (dashed line in Fig. 2).

$$ DNA + H_{\text{site2}} \xrightarrow{k_{\text{obs}}^{\text{two}}} DNA-H_{\text{site2}} \quad (4) $$

$$ \Delta m_t = \Delta m_{\text{max1}} \{1 - \exp(-k_{\text{obs1}} t)\} + \Delta m_{\text{max2}} \{1 - \exp(-k_{\text{obs2}} t)\} \quad (5) $$

Figure 3 shows the plots of the $k_{\text{obs}}$ against DNA concentration, with a linear least-squares method applied to fit the data. According to Eq. (3), $k_1$ is derived from the slope of line and the value of $k_{-1}$ corresponds to the ordinate intercept in Fig. 3. The QCM analysis yielded values for $k_1$ of $0.8 \text{ s}^{-1} \text{mM}^{-1}$ and $k_{-1}$ of $0.001 \text{ s}^{-1}$, whilst values for $k_1$ of $1 \text{ s}^{-1} \text{mM}^{-1}$ and $k_{-1}$ of $0.002 \text{ s}^{-1}$ were derived from the SPR data. The association constant ($K_a$) can be calculated by dividing $k_1$ by $k_{-1}$, giving $K_a$ of $8 \times 10^5 \text{ M}^{-1}$ and $5 \times 10^5 \text{ M}^{-1}$, respectively. Considering measurement deviation, the rate constants obtained from two different techniques (QCM and SPR) can be regarded as approximately the same. Additionally, values for $K_a$ obtained from other studies (1.2–10$^5$–10$^6$ M$^{-1}$) are similar to the values obtained from this study [9, 10].

Figure 4 shows the time courses of the change in frequency during QCM analysis in response to the addition
FIG. 4. Time courses of the frequency change during QCM in response to the addition of DNA solution into a cell without immobilized PEI.

FIG. 5. Model fitting for QCM measurements after addition of DNA solution to a cell without immobilized PEI.

FIG. 6. The plots of the $k_{obs2}$ (or $k_{obs}$ for observations without PEI) versus DNA concentration. The frequency change observed in the absence of PEI increased in a DNA-concentration dependent manner. Under these conditions, the single site model fitted best with the frequency results obtained and was therefore used to derive a value for $k_{obs}$ (Fig. 5).

IV. CONCLUSIONS

In conclusion, we determined the rate constants for binding between DNA and PEI using QCM and SPR. We applied a two-site model to fit the data and found that DNA appeared to bind to the gold electrode surface as well as to the PEI. As the $K_a$ values obtained from this study were similar to the values obtained by other measuring techniques, we consider that the methods evaluated in this study are valid. The two-site fitting model we propose might be widely applicable to binding reactions that include the potential for other non-specific binding reactions.