Quantitative Detection of Protein Binding onto DNA by Using a Quartz-Crystal Microbalance

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(Received June 21, 1996)

Selective binding process of proteins to DNA strands immobilized on an Au electrode of a quartz-crystal microbalance (QCM) was observed *in situ* from frequency decrease (mass increase). The binding kinetics of sequence specific binding of NFI to DNA containing its recognition site were compared with those of non-specific binding of histone f3.

It is vitally important to understand the nature of protein-DNA interactions, as they are intimately linked to the control of gene expression.[1] For example, Nuclear factor I (NFI) is one of well-understood sequence specific DNA binding protein isolated from uninfected HeLa cells, which binds selectively to the DNA sequence of $TGGN_{6\text{-}7}GCCAA$.[2] These interactions have been qualitatively or semi-quantitatively studied by using filter binding assay[2] and gel shift assay.[2,3] These conventional methods have some difficulties: (i) pre- or post- treatments are required to modify DNA or proteins by introducing fluorescent- or radioisotope- labels; (ii) it takes relatively long time to obtain the results; and (iii) it is difficult to detect *quantitatively* the absolute binding amount and binding kinetics.

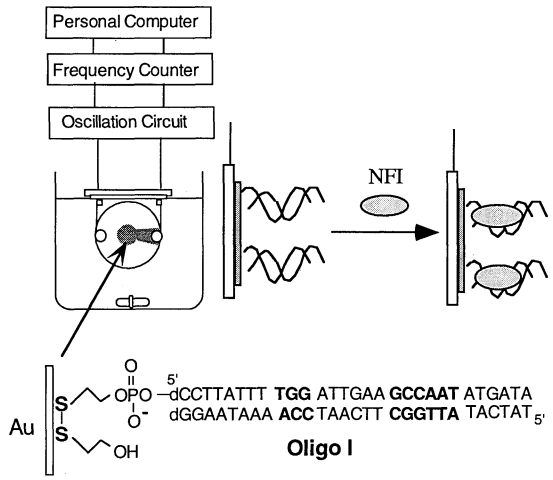

Figure 1. Schematic illustration of sequence specific binding of Nuclear factor I (NFI) protein onto the Oligo I DNA strand immobilized on a 9 MHz QCM. Bold letters in the Oligo I DNA strand show the specific binding-site for NFI protein. dA_{30}-dT_{30} DNA strand was also immobilized for detecting nonspecific binding of histone f3.

In this communication, we propose a new methodology to detect, *in situ* and *quantitatively*, binding processes of proteins to DNA strands immobilized on Au electrode of a quartz-crystal microbalance (QCM) from its frequency change (see Figure 1). QCMs are a useful device to detect molecular binding process because their resonance frequency decreases upon the increase of a mass on the QCM on a nanogram level.[5-7] We chose two different proteins such as NFI showing high sequence specificity for the target DNA[2] and histone f3 showing simple electrostatic binding independent of DNA sequences.

The QCM employed in this study is commercially available 9 MHz, AT-cut quartz, on both sides of which Au electrodes were deposited (area: 16 mm^2 x 2). The one side of the QCM was covered with a rubber case to avoid the contact with the ionic buffer solution. The QCM was connected to a handmade oscillator designed to drive the quartz at its resonance frequency in aqueous solutions.[7] The frequency changes were followed by a universal frequency counter (Iwatsu Co., Tokyo, model SC 7201) attached to the microcomputer system (NEC Co., Tokyo, model PC 9801). Calibration showed that a frequency decrease of 1 Hz corresponded to a mass increase of 3.0 ng on the QCM electrode (16 mm^2) in aqueous solution (see also Figure 2).[5,7]

The 29-mer deoxynucleotide (Oligo I DNA)[2] having a recognition site of NFI protein ($TGGN_{6\text{-}7}GCCAA$) and 5'-phosphate end-group was prepared by the phosphoramidite method,[8] and β-hydroxyethyldithioethoxy group was introduced to the 5'-phosphate group by using water-soluble carbodiimide, and hybridized with its complementary 29-mer DNA having no end phosphate group. A non-specific DNA double strand of dA_{30}-dT_{30} was also prepared in the same manner, in which the S-S linkage was introduced to a dT_{30}. The QCM having Au electrodes on both sides was immersed into an aqueous solution (0.3 M NaCl) containing double-stranded DNA having a S-S-group (1 mg DNA/mL) at 25 °C.[9] The immobilized amount of the probe DNA was followed by measuring the frequency decrease (mass increase) with time, and the QCM was picked up after 1 h when 10 ± 2 ng (35 pmol) of the probe was immobilized on one side (16 mm^2) of Au electrode. This value was calculated to be *ca.* 10% coverage of the Au electrode surface of the QCM. The immobilized DNA was physically and chemically stable on the electrode for many months and for washing with aqueous solutions.

Figure 2 shows typical time courses of frequency changes of DNA-immobilized QCM responding to the addition of proteins in a solution of 25 mM HEPES buffer (pH 7.5), 70 mM NaCl and 1 mM EDTA at 25 °C. When NFI (0.45 nM) was injected into the Oligo I DNA-immobilized QCM, the frequency decreased with time and reached equilibrium within 30 min. When dA_{30}-dT_{30} Oligo DNA was immobilized on a QCM, NFI hardly bound. When histone f3 was injected even at ten times higher concentration (4.5 nM), it bound slowly and non-selectively to both the Oligo I and dA_{30}-dT_{30} DNAs. When bovine serum albumin (slightly negatively-charged) was injected, it hardly bound to both the Oligo I and the dA_{30}-dT_{30} DNAs. These results indicate that NFI can bind selectively to the Oligo I DNA having a $TGGN_{6\text{-}7}GCCAA$ sequence, but not to non-specific DNAs. On the contrary, positively charged histone f3 can bind to both DNAs irrespective of their sequences possibly due to electrostatic interactions.

The binding amount of NFI (Mw: 4.5 x 10^4) was found to be 32 ± 5 ng as seen in the frequency decease of Figure 2. NFI was calculated to bind to *ca.* 70% of the Oligo I DNA (Mw: 1.9 x 10^4) of which 10 ± 2 ng were immobilized on the QCM, *ca.* 10% coverage of its surface.

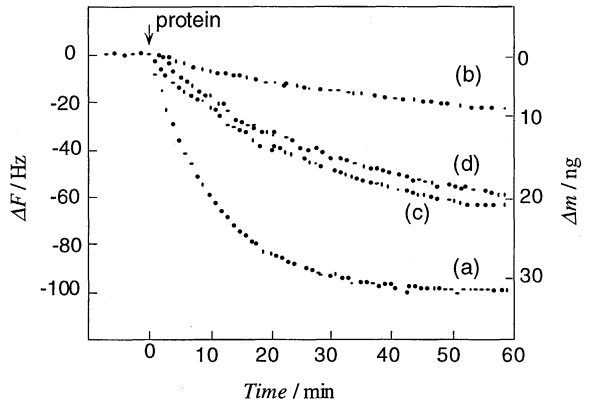

Figure 2. Time courses of frequency decreases (ΔF) or mass increases (Δm) of the DNA-immobilized QCM (a): Oligo I DNA with NFI, (b): dA_{30}-dT_{30} DNA with NFI, (c): Oligo I DNA with histone f3, and (d): dA_{30}-dT_{30} DNA with histone f3 (25 °C, pH 7.5, 25 mM HEPES buffer, 70 mM NaCl, 1 mM EDTA, [NFI] = 0.45 nM, [histone f3] = 4.5 nM).

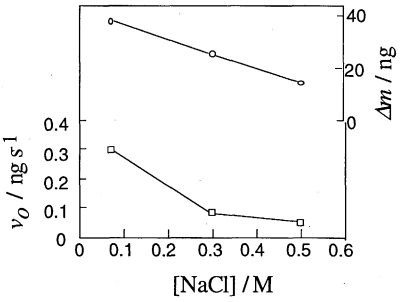

Figure 3. Effect of ionic strength on NFI binding to the Oligo I DNA at 25 °C.

Figure 3 shows effect of ionic strength on sequence specific NFI binding to the Oligo I DNA. Both initial binding rate (v_0) obtained from the initial slope of Figure 2 and equilibrium binding amount (Δm) decreased with increasing NaCl concentration in the aqueous solution from 70 to 500 mM. This suggests that the main motive force of NFI binding to the Oligo I DNA is electrostatic interaction and sequence specificity should be observed by hydrogen bonding between peptides and nucleic bases.[10]

The time course of binding behavior of proteins onto DNA in Figure 2 is expressed by eq. (1):

$$[\text{DNA}] + [\text{Protein}] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{DNA/Protein}] \quad (1)$$

The amount of the DNA/Protein complex, formed at time t , is given by eq. (2):

$$[\text{DNA/Protein}]_t = [\text{DNA/Protein}]_\infty\{1 - \exp(-t/\tau)\} \quad (2)$$

where $[\text{DNA/Protein}]_t = \Delta m_t$, $[\text{DNA/Protein}]_\infty = \Delta m_\infty$, and $1/\tau = k_1[\text{Protein}] + k_{-1}$. The binding and dissociation rate constants (k_1 and k_{-1}, respectively) were obtained from eq. (2) at four different concentrations of proteins (0.1, 0.2, 0.3, and 0.4 μg in 5 ml). Association constants (K_a) were obtained from k_1/k_{-1}, and kinetic parameters are summarized in Table 1.

Table 1. Kinetic parameters for binding of proteins onto DNAs immobilized on a QCM at 25 °C[a]

DNA	Proteins	k_1 / 10^5 $M^{-1}s^{-1}$	k_{-1} / 10^{-5} s^{-1}	K_a / 10^9 M^{-1}
Oligo I	NFI	16	79	2.0
dA_{30}-dT_{30}	histone f3	1.1	7.0	1.5

[a]Data contain ±10% experimental errors.

Sequence selective binding of NFI to the Oligo I DNA showed *ca.* 10 times larger binding rate constant (k_1) and dissociation rate constants (k_{-1}) than those of non-specific binding of histone f3 to the dA_{30}-dT_{30} DNA. Consequently, the sequence specific binding of NFI and non-selective binding of histone f3 showed similar association constants ($K_a = 10^9$ M^{-1}).

In conclusion, the DNA-immobilized QCM will become a useful technique to detect directly the protein-DNA interaction and its binding kinetics.

References and Notes

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