The Length Effect of Probe DNA for Hybridization using DNA Self-Assembled Monolayer*

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To establish a DNA sensor, DNA self-assembled monolayer (SAM) was prepared on a gold substrate. A probe DNA SAM was fabricated using thiolated DNA composed of single- and double-stranded portions on a gold substrate. The adsorption behaviors of probe DNAs were measured by surface plasmon resonance (SPR). Hybridization efficiency was optimized by controlling the length of spacer inserted in probe portion and SAM layer. Hybridization with target DNA on the DNA array prepared using probe DNA was monitored in situ by SPR imaging. The SPR image indicates that 10-thymine bases spacer shows the most efficient hybridization on the DNA array. [DOI: 10.1380/ejsnt.2005.250]

Keywords: DNA array; Self-assembled monolayer; Hybridization; Thymine spacer; Surface plasmon resonance imaging

I. INTRODUCTION

The hybridization of poly- or oligo-nucleotides, such as DNA or RNA, is generally used in gene analysis. Recently, DNA microarrays, such as a DNA chip, are being used for high-throughput analysis [1, 2]. At present, the sensitive detection of target DNA is strongly required in medical fields because only a small amount of target DNA can be obtained from patients for the assay. Although polymerase chain reaction (PCR) is generally utilized to amplify the target DNA, the profile of gene expression might be changed after the PCR amplification.

Many attempts about immobilization of probe DNA on a solid surface were reported previously [3, 4]. We reported the preparation method for DNA self-assembled monolayer (SAM) on a gold substrate using a probe DNA composed of single-stranded (ss) and double-stranded (ds) oligonucleotide portions, as shown in Fig. 1. The probe DNA forms SAM on a gold substrate by addition of divalent Mg ion into the solution [5–7]. The unique architecture of this probe DNA allows for relatively close packing of the lower section of the DNA SAM on the surface whereas in the upper section sufficient free space exists for DNA hybridization. To establish highly sensitive DNA sensors, it is essential to optimize the activity of the probe DNA on the surface. In this study, we focused on the mobility of probe portions on the substrate. The length of spacer inserted between the probe portion and the ds portion was varied to control the mobility of the probe portion on the surface. The chemical bonding of the thiol group with gold was induced to immobilize the probe DNA on a gold substrate and the formation of the monolayer was carried out by a self-assembly technique. SAMs have attracted considerable

attention for well-ordered thin-film fabrication, by which thiol or disulfide derivatives can spontaneously form a closely packed monolayer on a gold surface when the substrate is immersed in a thiol or disulfide derivative solution [8–10]. We observed the adsorption of probe DNA onto a gold substrate surface plasmon resonance (SPR). The hybridization with target DNA on the DNA array prepared using probe DNAs was observed by SPR imaging to optimize the length of the spacer for the efficiency of the hybridization.

II. EXPERIMENTAL

A. Materials

Table 1 shows the nomenclature and sequences of the probe and target DNAs used in this study, which were purchased from JBIOS. (Saitama, Japan). The preparation of probe DNA was described elsewhere [5]. To prepare the probe DNAs, ss 40–70 oligonucleotides and thiolated 20 oligonucleotide in their equimolar solutions were annealed up to 95°C, to form ds DNA in the complemen-

FIG. 1: Preparation of probe DNA containing ss and ds portions.

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TABLE I: Nomenclature and sequences of DNA. Underlined portion shows probe portion hybridizing with target DNA.

Name	Probe DNA
20merSH	3'-ATCGTACGATTACGTACGTA-5'-C6-SH
40-70mer	5'- <u>TGGAGAACTGATCGACACAG</u> -(T) ₀₋₃₀ -TAGCATGCTAATGCATGCAT-3'
20merC	5'-TAGCATGCTAATGCATGCAT-3'
Target DNA	
20mer	5'-CTGTGTCGATCAGTTCTCCA-3'
Probe DNA Complex	
Probe (0)	40mer/20merSH
Probe (10)	50mer/20merSH
Probe (20)	60mer/20merSH
Probe (30)	70mer/20merSH
Blocking DNA	20merC/20merSH

tary region. (Fig.1) The formations of the complexes were confirmed by electrophoretic analysis. Every probe DNA has a spacer of 0-30 thymine bases between the probe and ds portions to control the mobility of the probe portion. The probe DNAs were dissolved in a sterilized aqueous 20 mM MgCl₂·6(H₂O) solution (MgCl₂ solution) to a final concentration of 2.7 μ M. Those probe DNA SAMs were prepared by immersing a gold substrate into the aqueous MgCl₂ solution containing the probe DNAs for 15 hours. Ss 20-bases oligonucleotide complementary to the probe portion was used as a target DNA. The target DNA was dissolved in 5 $\times$ SSC buffer (Sigma, [Na⁺]=750 mM) to a final concentration of 3.0 μ M.

B. SPR measurements

To monitor reactions occurring at the solid-liquid interface, SPR was employed in the detection of the adsorption of the probe DNA onto gold substrates. SPR measurement (NanoSensor, Nippon Laser Electronic, Japan) was carried out on the gold substrate, which was prepared by the vacuum deposition of gold onto a glass (BK-7), and the thickness of the gold was $\sim$ 50 nm. The temperature of the SPR cuvette was controlled at 20°C. The flow rate of sample injection and rising was controlled at 0.1 ml/min with a flange pump (P.C.S. PUMP, Model 021, Toyobo, Japan).

C. Observation by SPR imaging

SPR imaging is a promising method for high-throughput detection of binding events occurring on a surface. SPR imaging measurements were carried out by SPRImager (OWG Instruments, Wisconsin, US). Figure 2 shows the experimental setup for SPR measurements. Incoherent light over the wavelength range of 600-1100

FIG. 2: Detection of DNA hybridization on DNA array by SPR imaging.

FIG. 3: Adsorption behaviors of probe DNAs onto gold substrates by SPR.

nm was used as a source. The details of SPR imaging observation were described previously [11-13]. A CCD camera was used to collect the images, were examined using the software package V++ (Digital Optics, New Zealand) on a personal computer. A probe DNA array was prepared on the gold substrate as follows. DNA spots were prepared on the gold substrate by an automated spotter (MultiSPRinter, TOYOBO, Japan). The DNA array was prepared on a 50-nm-thick gold evaporated on a glass slide (SF-10) that were covered first with a 2-nm-thick chromium layer to increase mechanical stability. After spotting the probe DNA, the array was incubated at 37°C in an oven for 16 hours and then rinsed with excess aqueous MgCl₂ solution containing thiolated ds 20 base pairs oligonucleotides (**Blocking DNA**, approximately 100 μ l, 3.0 μ M) to prepare a ds DNA SAM in the space between the spots on the gold substrate. The **Blocking DNA** was prepared by annealing of the equimolar mixed solution of 20 merC and 20 merSH. This ds DNA SAM has a blocking effect, which decreases the nonspecific adsorption of target DNA to the areas between the spots, hence leading to high-contrast images. The flow rate of sample injection and rising was controlled at 0.1 ml/min with the same flange pump as mentioned above. The image collected by the SPR imaging measurement was further analyzed using the SPIP software (Image Metrology, Denmark).

FIG. 4: Fig.4 (a) Layout of DNA array containing four kinds of probe DNAs and SPR difference image of the array after hybridization. (b) Summary of hybridization efficiency on the spots prepared using the probe DNAs.

III. RESULTS AND DISCUSSION

A. Formation of probe DNA SAM on a gold substrate

The details about the formation of DNA SAM using the **Probe (10)** onto a gold substrate are described elsewhere [5]. When the MgCl_2 solution was used for the solvent, the thickness of DNA SAM was remarkably change while only a small amount of the probe DNA adsorbed onto the gold substrate in the TE buffer. The DNA SAM prepared using **Probe (10)** was well characterized by SPR and XPS. Figure 3 shows adsorption behaviors of the probe DNAs onto the gold substrates by SPR. These results indicate that each probe DNA forms a stable monolayer on a gold substrate as well as **Probe (10)** does. Intriguingly, there is no significant difference in the adsorption signal of **Probe (0)**, **Probe (20)** and **Probe (30)**, whereas the signal of **Probe (10)** is lowest among them. If the molecular orientation in the every monolayer prepared from the probe DNA were uniform, the thickness of layer would be in proportion to the number of bases. These SPR results implied that the orientation of ds portions might be different in each SAM. In particular, in the case of the probe DNA containing the longer ss portion, most of them might

adsorb on the substrates via non-specific adsorption because nucleic bases of ss portions have an affinity to a gold substrate. The formation of SAM might be bound up with the length of ss and ds portion in the probe DNA.

B. Observation of hybridization with target DNA using a DNA array

After the preparation of the DNA array containing the four kinds of probe DNA, SPR imaging measurements were carried out to monitored the hybridization with target DNA on the DNA array. The left illustration and the right image in Fig. 4 (a) show a layout of DNA array composed of four kinds of probe DNA prepared on a gold substrate and SPR image after hybridization with target DNA, respectively. The SPR image was taken by subtracting the SPR images before and after the hybridization. Each probe DNA has four spots on the same substrate to confirm the reproducibility of the hybridization. In this image, the SPR signal was observed on the spots prepared using **Probe (0)**, **Probe (10)** and **Probe (20)**. On the other hand, the spots prepared from **Probe (30)** showed little signal even after the hybridization assay. To discuss quantitatively, the hybridization efficiency estimated by the SPR imaging is summarized in Fig. 4

(b). The evaluated values of the hybridization signal corresponding to the brightness of SPR images were taken on the average. The spots prepared from the **Probe (10)** showed the best result among them, while the hybridization signal was hardly observed on the spots prepared from the **Probe (30)**. In the cases of **Probe (0)** and **Probe (20)**, both signals are approximately 40% in comparison with the case of **Probe (10)**. The hybridization signal should be increasing with the increment of the spacer length at the viewpoint of mobility of the probe portion if the only mobility of the probe portion controls the hybridization efficiency. Although this unexpected result is still under discussion, there are two possibilities to explain the SPR imaging result: one is a dissociation of ds portion was accelerated by the flexibility of the long spacer, then hybridization signal was decreased on the spots prepared by **Probe (30)**. The hybridization efficiency is governed by the equilibrium of association and dissociation of two complementary parts of nucleotides. In this case, the SAM prepared using **Probe (10)** showed the best condition of the mobility of probe portions and the stability of ds formation after hybridization. Another possibility is that the activity of probe portion was decreased because of denaturation of the probe portions on the substrate. In the second case, the **Probe (30)** did not form a stable monolayer on a gold substrate and most

of probe portions of **Probe (30)** might be denatured on a gold substrate. The result of adsorption of probe DNAs implied that the orientation of each probe DNA was not uniform on the substrate.

IV. CONCLUSION

In this study, probe DNAs containing various length of spacer were employed to optimize the efficiency of hybridization. Adsorption behaviors of probe DNAs monitored by SPR suggested the formation of monolayer on gold substrates. The SPR image on a DNA array suggested that the probe DNA SAM prepared using **Probe (10)** showed the most efficient hybridization. Because the molecular design of probe DNA is indispensable for the highly sensitive DNA sensors, these results will become significant information in a development of DNA sensors.

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