Surface Plasmon Resonance Imaging of Au Nano-Particle Modified DNA Monolayers

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ABSTRACT

A probe DNA monolayer modified with Au nano-particles was prepared on an Au thin film and used for DNA hybridization detection by surface plasmon resonance (SPR) spectroscopy. This Au particle modified probe DNA monolayer resulted in about a 3.5 times larger SPR angle shift according to the hybridization reaction, compared to that found in a non-modified probe DNA monolayer. By comparison of the SPR image of the particle modified and non-modified probe DNA monolayer, this modification method was shown to be useful in improving the SPR signal for the detection of the unlabelled DNA molecules.

Keywords: DNA, Hybridization, SPR, Au nano-particle, AFM

1. INTRODUCTION

Surface plasmon resonance (SPR) spectroscopy is a simple and easily performed optical method for observing DNA hybridization adsorption at metal interfaces.1 SPR spectroscopy is based on the detection of changes in the SPR angle (angle of incidence at minimum reflectivity) induced by a small change of the refractive index at the metal surface.1 It allows detection of the hybridization adsorption of unlabeled target DNA in situ. SPR spectroscopy has been used for monitoring the adsorption and hybridization of DNA at solid/water interfaces,2,3 and for simultaneous imaging of array of spotted some probe DNA.4-7 Despite the many applications of SPR spectroscopy, problems often arise in detecting small shifts of the SPR angle in small organic adsorbents, such as DNA molecules. For the purpose of increasing its accuracy and detection limits, some efforts have been made to enhance the SPR signal; for example by binding a colloidal Au particle6 and large molecules such as streptavidin.4 Since these methods are based on the addition of an extra refractive index or large mass to the target DNA, they require the modification of the target molecules before and/or after the hybridization reaction; thus the advantages of SPR spectroscopy are lost.

In our previous work, it was demonstrated that the modification of the probe DNA monolayer by the Au nano-particle could enhance the SPR angle shift caused by hybridization with target DNA molecules.8 This method requires no modification of the target DNA, and no process for signal enhancement after the hybridization reaction. Therefore, it is expected that this method can be applied for the simple detection of unlabelled DNA molecules by SPR imaging measurement. In this work, we examined the SPR image of the particle modified and non-modified DNA monolayers.

2. EXPERIMENTAL

2-1. MATERIALS

The DNA was purchased from Nisshinbo. The DNA sequences are listed in Table 1. The ss-probe DNA (BD), of which the 3 phosphate end was mono-esterified with (HO-(CH2)2-S-)2, was used to form the ss-DNA monolayer on the Au film. BD has a biotin at the 5 end of the DNA as a phosphate ester with an alkyl spacer. T1 and T2 were used for the complementary or non-complementary target DNA molecules. Au particles (diameter = 9.0–0.7 nm) coated by

straptavidin-albumin were purchased from SIGMA. KH₂PO₄ and K₂HPO₄ were obtained from Kanto Chemical. NaCl was obtained from Aldrich, and the other reagents were purchased from Dojindo. The water used in all of the experiments was Milli-Q water.

**TABLE 1. SEQUENCES OF DNA OLIGONUCLEOTIDES USED.**

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>BD</td>
<td>5' biotin CACGACGTTGTAAAACGACGGCCAGATCAT 3'</td>
</tr>
<tr>
<td>TI</td>
<td>ATGATCTGGCGCTGTTTTACAACGTGTG</td>
</tr>
<tr>
<td>T2</td>
<td>AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA</td>
</tr>
</tbody>
</table>

2-2. PREPARATION OF DNA MONOLAYERS

For atomic force microscopy (AFM) and SPR reflectivity curve measurements, quartz substrate (Pier Optics) was used as sample substrates. The cleaned quartz substrate was modified with 3-aminopropyltriethoxysilane in order to enhance the adhesion of the Au thin film to the quartz substrates. The cleaned substrate was immersed in 10% (v/v) 3-aminopropyltriethoxysilane ethanol solution and rinsed with ethanol and water, then blown in a nitrogen stream. These immersing and rinsing procedures were repeated twice. The Au thin film was sputtered by a sputtering system (ULVAC) at room temperature. The thickness of the Au film was monitored by a quartz crystal thickness monitor (ULVAC). After sputtering, the film was immediately annealed in the sputtering chamber at 300 °C for 3 min under a high vacuum (ca. 2.0×10⁻⁴ Pa), in order to decrease the surface roughness. In the SPR imaging measurement, a cover glass (Matsunami) plate was used instead of the quartz substrate, and a Cr layer (thickness = ca. 1 nm) was employed to enhance the adhesion of the Au thin film. The thin Au films were stored in a clean plastic box filled with N₂ gas, and immersed in concentrated sulfuric acid solution before use.

The DNA monolayers were prepared according to the following procedures. 3 μM (M = mol dm⁻³) of ss-probe DNA solution (0.5 M KH₂PO₄ and 0.5 M K₂HPO₄, pH 7.0) was dropped on the substrate and stored in a humid atmosphere for 14 h. After rinsing, the colloidal Au solution was dropped onto the ss-probe DNA monolayer. After rinsing with the Tris buffer solution (10 mM NaCl and 5 mM Tris-HCl, pH 7.4), the modification of the Au particles in relation to the ss-probe DNA monolayers was performed by exposure to the streptavidine-albumin coated colloidal Au solution (10 mM NaCl and 5 mM Tris-HCl, pH 7.4) for 3h. After rinsing with the Tris buffer, hybridization was performed at room temperature in a 10 μM target DNA solution for 16 h. The hybridization reaction was performed in 1.0 M NaCl with 10 mM Tris buffer, pH 7.4, and 1 mM EDTA. Finally, the sample substrates were rinsed with the Tris buffer and dried under a moderate stream of nitrogen.

2-3. MEASUREMENTS

The SPR reflectivity curves were measured using a linearly polarized He-Ne laser beam. The polarization of the laser beam was set parallel to the plane of incidence (p-polarized) by a λ/2 wave plate and Glann-Thompson polarizer (Melles Griot). The polarized laser light was focused onto the sample substrate through a 45° prism, contacting the sample substrate through the index matching fluids. The reflected light was detected by a photomultiplier tube (HAMAMATSU: Model H5784) and the signal was processed by a lock-in amplifier.

The schematic of an SPR imaging apparatus is shown in Figure 1. The He-Ne laser was used as a light source. The light shape was arranged using 10× objective lens (Nikon, Plan Fluor), 100 μm pinhole and a Lens. The polarization of the incident light was set with a Glann-Thompson polarizer and Fresnel Rhomb (SIGMA). The reflected light from the sample substrate was captured by a charge coupled device (CCD) camera (Sony, Model DXC-151A).

Atomic force microscopy (AFM) measurements were performed using a SEIKO SPA 300 microscope in the non-contact mode. A silicon cantilever with a spring constant of 1.8 N/m was used. In order to confirm the surface density of the Au particles, the images were obtained three to four positions apart from each other by 1 to 2 mm for each monolayer, and the measurements were performed using three samples prepared at different times.
3. EXPERIMENTAL RESULTS AND DISCUSSION

3-1. CHARACTERIZATION OF THE AU PARTICLE MODIFIED SS-PROBE DNA MONOLAYER

The Au particles adsorbed on the BD monolayer were observed by AFM (Figure 2). We were not able to observe particle multi-layers in the AFM images by narrowing the scan range, and the height of the particles of about 9 nm were estimated from the height analysis. These results indicate that the BD monolayer is modified with the Au particles by biotin-avidine interaction. Counting the particles in the AFM images indicated about 15 particles in the 10000 nm² region. On the other hand, we had estimated the surface density of the BD monolayer as being 14 nm² in our previous study. Therefore, it appears that most BD molecules were not attached to the Au particles, and that the BD monolayers can be regarded as a spacer layer between the Au particles and the film surface.

Figure 2. AFM images of Au particles modified ss-probe DNA monolayer. The scan area is 1 μm × 1 μm.

3-2. SPR RESPONSE OF AU NANO-PARTICLE MODIFIED DNA MONOLAYER

Figure 3 shows the SPR response of the DNA monolayers. In the absence of Au particles, the SPR angle shift before/after the hybridization with a complementary target (T1) was 0.12°. When the probe DNA monolayer was modified by the Au particles, the SPR angle shift before/after hybridization became 0.42°, about 3.5 times larger than the 0.12° found in the non-modified monolayers. We could not observe a significant angle shift in the Au particle modified probe DNA monolayer after exposure to the T2 solution. The T2 is not expected to make a duplex with the probe DNA molecules. Therefore, it was considered that the larger SPR angle shift was due to the hybridization of T1 with the probe DNA molecules.

As reported in previous papers, a large SPR angle shift is induced by a metallic nano-particle in the vicinity of metallic thin films, and the angle shift depends on a refractive index surrounding the particles, and a nano-meter order separation between the particles and the film surface. In our experimental conditions, the DNA monolayers can be regarded as a spacer layer between the Au particles and the film surface. The refractive index of the ss-DNA monolayer changes by the duplex formation, because the ds-DNA molecule has a greater amount of nucleotides than the ss-DNA.
It is also expected that an average thickness would be different between the ss- and ds-DNA monolayers, because the chain length of a 30-base ss-DNA molecule is about 20.4 nm, and that of a ds-DNA is 10.2 nm. Therefore, we consider that the characteristic (the refractive index and the layer thickness) changes of the ss-probe DNA monolayer, which are caused by the hybridization with the target DNA molecules, would be responsible for the larger SPR angle shift in the Au particle modified probe DNA monolayer.

Figure 3. SPR reflectivity curves for (A) non-modified and (B) Au particle modified DNA monolayers. Open circles and filled circles represent before and after hybridization with T1

The SPR image of the DNA monolayers was depicted in Figure 4. The intensity of the reflection light from the non-modified DNA monolayer became slightly stronger after hybridization with T1, but the intensity difference was not obvious. On the other hand, The Au particle modified probe DNA monolayer shows a clear difference before/after the hybridization reaction. Thus, the method of the Au particle modification to the probe DNA monolayer could improve the SPR signal for DNA hybridization detection.

Figure 4. SPR images of DNA monolayers. Incident angle was set at the SPR angle of bare Au surface.

4. CONCLUSION

The present experimental studies demonstrate that the modification of the probe DNA monolayer by the Au nanoparticles could be used for an improvement of DNA hybridization detection by SPR imaging measurement. This method has some possible advantages for DNA hybridization detection. No modification of the target DNA is required. The Au particle is not photo-bleached by probe light irradiation. Moreover, this method can be expected to be applied...
to an in-situ monitoring of the DNA hybridization and quantification analysis, because any process is not necessary for signal enhancement.

At our experimental conditions, most probe DNA molecules were not attached to the Au particles and the surface density of the Au particles was not saturated. Therefore, the SPR angle shift would be even more enhanced by increasing the amount of the Au particles attached to the probe DNA monolayer. In conclusion, this modification method has potential advantages and practical application for DNA hybridization detection, and promises easy and reliable gene analysis.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Education, Culture, Sports, Science, and Technology of Japan for a Grant-in Aid for Scientific Research A (2) (No. 1330518), and a Grant for the Collaboration between University and Society (No. 11793004). This work was also supported in part by the NEDO regional consortium project "Development of New Micro-Array for Gene Analysis," the Foundation Advanced Technology Institute, and the Satellite Venture Business Laboratory of the University of Tokushima.

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