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In-Situ Surface Infrared Study of DNA Hybridization on Au Island Films Deposited on Si Surfaces

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1. Introduction

DNA chips and microarrays are powerful tools that have been widely used for the analysis of single nucleotide polymorphisms and gene expression. These techniques are based on immobilization of single-stranded DNA, DNA hybridization and subsequent detection of fluorescent signals by a fluorescence microscope. Although this method has a quite high sensitivity of detection of reacted species, fluorescent tags might have some influence on the chemical and structural properties of DNA.

Infrared absorption spectroscopy (IRAS) provides an alternative method for detection of biomolecules. IRAS allows nondestructive and *in-situ* detection of biomolecules, and most importantly, it does not require any labeling, such as fluorescence or enzyme. Recently, we have proposed an *in-situ* method for detecting DNA hybridization at silicon (Si) surfaces by using IRAS in the multiple internal reflection (MIR) geometry.[1)] We demonstrated that conformational changes in DNA strands due to hybridization produces the specific C=O stretching vibration modes in the hydrogen-bonded base pair. However, a crosslinker used for immobilizing probe DNA also contained C=O bonds, which caused high background absorption around the C=O stretching vibration region, resulting in relatively high DNA concentration needed to produce clear spectral changes. As a platform for immobilizing DNAs, Au nanoisland films on Si prisms are attractive materials. Au-thiol chemistry enables designing DNA-immobilized surfaces with low background around the C=O stretching vibration region. Although Au is a material known to reflect IR radiation, such inhomogeneous Au island films allow evanescent field penetrated from the bare Si parts to the solution phase.

In the present study, we have investigated label-free IRAS detection of DNA hybridization on Au nanoisland films deposited on Si prisms (Fig. 1). Au island films were prepared onto Si surfaces by evaporation. DNA immobilization via Au-thiol chemistry and subsequent DNA hybridization was monitored with MIR-IRAS. We demonstrate that sensitive detection of DNA hybridization at 40 nM is achieved on Au island films deposited on Si by using MIR-IRAS. The present results show that MIR-IRAS is a powerful tool for sensitive label-free detection of DNA hybridization on Au island films, whose signal is based on the hybridization-specific conformational changes.

2. Experimental procedures

A rectangular Si prism (12 mm × 30 mm × 0.45 mm) was prepared from a double-side-polished, n-type Si(100) wafer with a resistivity of 1 - 10 Ωcm. The prism was polished with 45° bevels on each of the short edges. The surface of each Si prism was washed with water, followed by 1:1 H_2O_2:H_2SO_4 solution, 5 % hydrofluoric (HF) acid and water. The substrates were then loaded in a vacuum chamber for the deposition of an Au thin film. Au (99.95%, Nilaco, Japan) was evaporated from a tungsten boat. Scanning electron microscopy (SEM) images of the Au films thus prepared showed that the average diameter of the Au nanoislands was 5-10 nm (Fig. 2). The prism thus prepared was immersed into a solution of a 5'thiol-modified probe single-stranded

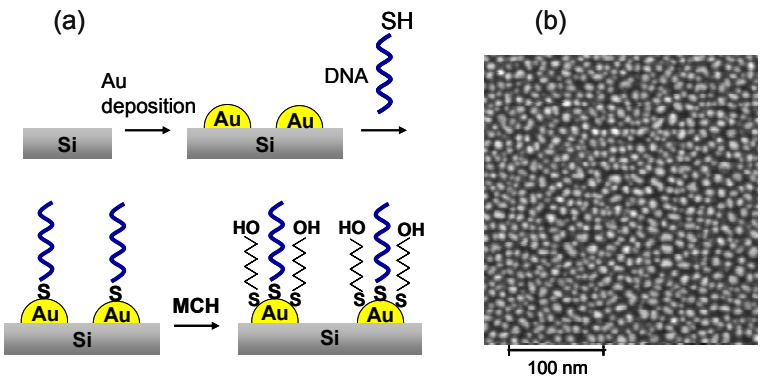

Fig. 1. (a) Schematic illustration of procedures for preparing DNA-modified surfaces. (b) A scanning electron microscopy (SEM) image of the Au films deposited on Si surfaces.

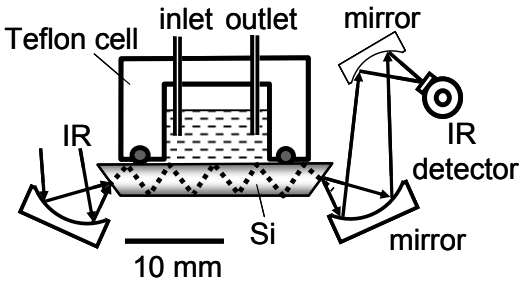

Fig. 2. A schematic illustration of the solution cell and configuration used in the present study.

DNA (5'-TTT TTT TTT TAAG CTG ATC CGT TAA CCT GA-3') for 2h. Then the surface was reacted with 1 mM 6-mercapto-1- hexanol (MCH) for 1h to block remaining active surfaces. Finally, a solution of 10 μM target DNA was added to the prism and incubated for 2h to hybridize with the immobilized DNA. In each step, the IR absorption spectra were collected. The same procedure was performed with noncomplementary DNA as a negative control. All the IRAS measurements and surface modification processes were performed in D_2O. The reason why we used D_2O instead of H_2O was as follows: H_2O has strong scissoring modes around 1650 cm^{-1}, where the bases of DNA have specific vibration modes that are quite sensitive to base-pairing. On the other hand, D_2O has no significant vibration modes at this frequency region.

2. Results and Discusstion

Fig. 3 shows typical IRAS spectra collected for the 20-mer complementary and noncomplementary target DNAs after reaction with the immobilized probe DNA. The probe DNA-modified surfaces exhibited absorption peaks in the frequency region of 1550-1750 cm^{-1}, which are due to the vibration modes of the bases. The dominant peak around 1660 cm^{-1} originates from the C=O stretching vibration modes of the nucleobases. Hybridization with complementary DNA (5'-TCA GGT TAA CGG ATC AGC TT-3') induced two distinct peaks at 1685 and 1644 cm^{-1}. On the other hand, when the noncomplementary DNA (5'-ACT GGA TGT CTG AAC TGT CA-3') was added to the surfaces, the spectrum exhibited no noticeable peak from 1550 to 1800 cm^{-1}. These results suggest that the increase in absorbance around 1685 and 1644 cm^{-1} is selective for DNA hybridization, which is in agreement with our previous observation that DNA hybridization induces increases in absorbance around 1690 and 1640 cm^{-1}.[1-3] It has been reported that spectral features located around 1690 cm^{-1} can be attributed to the C=O stretching modes of thymine and guanine residues.[4] We therefore suppose that this C=O stretching modes is affected by conformational changes due to DNA hybridization.

To explore the feasibility of the present method as a quantitative analysis, we then examined the DNA concentration dependence on the peak height at 1686 cm^{-1}. The peak intensity linearly increased with the concentration of the complementary DNA up to 100 nM. The lowest detectable concentration was 40 nM. This concentration is much lower, i.e. better, than those commonly used for IRAS detection.[1-3] The highly sensitive detection as IRAS could be achieved due to the MIR geometry combined with Au nanoisland films deposited on Si which provides low-background absorption around C=O stretching vibration region. Based on these results and consideration, we can conclude that MIR-IRAS combined with Au nanoisland films is useful for sensitive detection of DNA hybridization as well as providing information on conformational changes induced by DNA base-pairing.

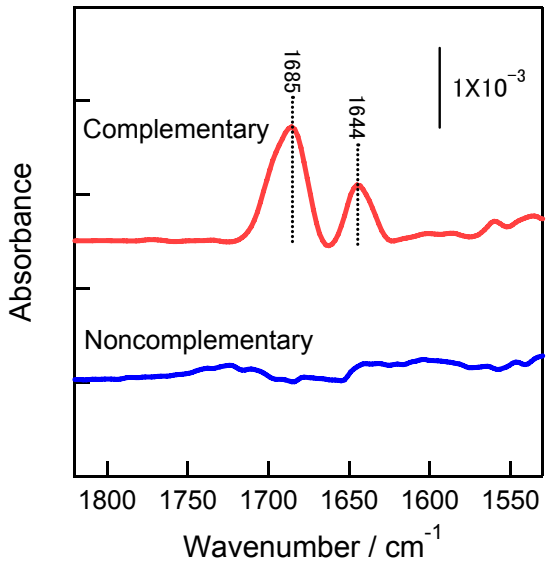

Fig. 3. The IRAS spectra of the DNA-modified Si surfaces and after reaction with target DNAs. Complementrary (top) and noncomplementry (bottom) DNAs. The reference was the spectra before addition of target DNAs.

3. Conclusions

We have investigated IRAS detection of DNA hybridization on Au nanoisland films deposited on Si surfaces by using MIR geometry. Sensitive non-label detection of 40 nM target DNA was achieved by using Au nanoisland films as a low-background platform.

Acknowledgements

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