**In-Situ Surface Infrared Study of DNA Attachment and Hybridization at Si Surfaces**

Ayumi Hirano, Kohki Tanaka, Kenichi Ishibashi, Koichiro Miyamoto, Yasuo Kimura, and Michio Niwano

1Research Institute of Electrical Communication, Tohoku University
2-1-1 Katahira, Aoba-ku, Sendai, 980-8577, JAPAN

**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. We have reported *in-situ* detection of hybridization of DNA in solution by IRAS in the multiple internal reflection (MIR) geometry. We demonstrated that conformational changes of DNA strands due to hybridization are reflected in the IRAS of the base part of DNA.

In this study, we have investigated DNA attachment on Si and subsequent hybridization using MIR-IRAS. Si surfaces were characterized through *in-situ* measurements of the IRAS spectra after each step of the surface modification and hybridization between the immobilized DNA (probe DNA) and its complementary DNA (target DNA).

**2. Experimental procedures**

Fig.1 schematically shows the reaction scheme for immobilization of single-stranded DNA on the Si surface. Si prisms (30x12x0.45 mm³) were polished with 45° bevels on each of the short edges and cleaned by the conventional RCA method. The surface of the prism was irradiated by ultra-violet (UV) lights to form silicon oxides. Then, the prism surface was covered with a self assembled monolayer (SAM) of 3-aminopropyl-triethoxysilane (APTES) in order to introduce an amine group on the surface. After that, a heterobifunctional crosslinker sulfo-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SSMCC) was attached to the surface of the prism. Finally, the prism was immersed into a solution of a 5’thiol-modified probe single-stranded DNA (5’-TTT TTT TTT TAAG CTG ATC CGT TAA CCT GA-3’) for 12 h. DNA Hybridization was performed in 2 M NaCl for 12 h at room temperature. Each step of the surface modification and DNA hybridization was examined by MIR-IRAS by using a Teflon cell as is schematically illustrated in Fig.2.

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**Fig. 1** The reaction scheme for immobilization of DNA on the surface of a Si prism.

**Fig. 2** A schematic illustration of the solution cell and configuration used in the present study.
depicted in Fig. 2. All the MIR-IRAS spectra were measured in D$_2$O. The reason why we used D$_2$O instead of H$_2$O was as follows: H$_2$O 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$_2$O has no significant vibration modes at this frequency region.

2. Results and Discussion

We first examined IRAS spectra of Si surfaces after the modification of APTES layer. APTES itself displayed the -CH$_3$ asymmetric vibration mode at 2974 cm$^{-1}$ and the -CH$_2$ symmetric and asymmetric vibration modes at 2885 cm$^{-1}$ and 2927 cm$^{-1}$, respectively. For the APTES SAM, peaks due to the -CH$_2$ symmetric (2893 cm$^{-1}$) and asymmetric vibration modes (2932 cm$^{-1}$) remained intact, while the -CH$_3$ vibration peak decreased significantly in intensity. This decrease can be interpreted as being due to the attachment of APTES, which is achieved by the removal of the methyl group (-CH$_3$) from the APTES molecule. The theoretically predicted spectra obtained using the ab initio cluster calculation exhibited similar results, supporting our interpretation.

IRAS spectra of the Si surface after immobilization of SSMCC exhibited two distinct peaks in the C=O stretching vibration region. Ab-initio cluster calculations showed that the peaks located at 1740 cm$^{-1}$ and 1699 cm$^{-1}$ are due to the C=O stretching vibration of maleimide and the coupling between the C=O stretching vibration of succinimide and that of an ester carbonyl. These spectral changes clearly indicate that SSMCC reacted with the SAM layer of APTES to produce a maleimide-activated surface, which can further react with thiol-modified oligonucleotides.

IRAS spectra of DNA-immobilized Si surfaces before and after hybridization are shown in Fig. 3. The reference was the spectrum collected for the SSMCC-modified surface. Two broad peaks at 1624 and 1661 cm$^{-1}$ before hybridization (dotted line) are mainly due to the C=C, C=O and C=N stretching vibration modes of the DNA bases.$^{1,2,3}$ This spectrum was similar to that obtained for a floating probe DNA in D$_2$O, indicating that the thiol-modified probe DNA was successfully immobilized on the SSMCC-modified Si surface. After hybridization with the complementary DNA (5’-TCA GGT TAA CGG ATC AGC TT-3’), notable enhancement in absorbance around 1685 cm$^{-1}$ was observed. When noncomplementary DNA was reacted with the immobilized probe DNA, the spectrum exhibited no noticeable peak around 1685 cm$^{-1}$. These results suggest that the increase in absorbance at 1685 cm$^{-1}$ is selective for DNA hybridization, which is in agreement with our previous observation that DNA hybridization induces an increase in absorbance around 1690 cm$^{-1}$.$^{1,2}$ 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.$^{3}$ We therefore suppose that this C=O stretching modes is affected by conformational changes due to DNA hybridization. Based on these results and consideration, we can conclude that MIR-IRAS is useful for detecting hybridization of DNA as well as providing information on conformational changes of DNA immobilized on Si surfaces.

3. Conclusions

We have investigated in-situ the process of DNA immobilization and subsequent hybridization on Si surfaces using MIR-IRAS. The Si surface after each modification step was well characterized by using MIR-IRAS. Hybridization experiments revealed that MIR-IRAS facilitates the detection of DNA hybridization through infrared spectral profiles in the frequency region around 1690 cm$^{-1}$. The present results show that MIR-IRAS is a powerful tool for the label-free detection of DNA hybridization as well as the in-situ (in vitro) characterization of conformation changes of DNA molecules immobilized on Si surfaces.

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