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In-situ Monitoring of DNA Hybridization Using Surface Infrared Spectroscopy

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Introduction

In the field of genomics, the gene expression has attracted much attention since it is closely related to some common diseases. The conventional method of analyzing the gene expression is fluorescence microscopy in which the base sequence of mRNA extracted from living cells and tissues is analyzed through the DNA hybridization and fluorescent signals. "DNA chip" and "DNA microarray" use this method and were widely used as a powerful tool that realizes a parallel analysis for thousands of samples [1]. In this method, however, fluorescence labeling of biomaterials is necessary which may have influence on the biological function of DNA. Infrared absorption spectroscopy (IRAS) provides an alternative method for analyzing the function of biomolecules. The advantage of IRAS over other methods is that IRAS is a nondestructive, non-contact method, and furthermore, fluorescence or radioactive labeling is not necessary. However, most previous studies of IRAS have not focused on a sensor application [2].

Recently we proposed a novel method to detect the DNA hybridization in solution using IRAS in the multiple internal reflection geometry (MIR-IRAS). MIR-IRAS uses Si prism as a waveguide and it is suitable to high sensitive observation of biomolecules on Si prism surface. MIR-IRAS is capable of detecting the DNA hybridization in solution as spectral changes [3, 4].

Experiment

[MIR-IRAS measurements] Figure 1 illustrates the electrochemical cell we used in this study for MIR-IRAS measurements. The setup was almost the same as used in our previous works [5,6]. The volume of the sample solution was 100-150 µl. A Si prism was 0.5 x 10 x 30 mm^3 with 45° bevels on each of the short edges, and contacts with the sample solution. An infrared light beam from an interferometer (BOMEM MB-100) was focused at normal incidence onto one of the two bevels of the Si prism, and penetrated through the Si prism, internally reflecting about 60 times. The light that exited the Si prism through the other bevel was focused onto a liquid-nitrogen cooled Mercury-Cadmium-Telluride (MCT) detector.

[DNA samples] We used two types of 30-mers oligonucleotides (ss-DNAs) that were by Nihon Gene Research Laboratories Inc., Sendai, Japan. We denote the oligonucleotides as L and R. The base sequence of oligonucleotide L was 5'-GGAG ACTG TTAT CCGC TCAC AATT CCAC AC-3'. R was complementary to L. These single-stranded oligonucleotides were solved in a solution of NaCl (typically 14.3 mM) in heavy water, D_2O. The concentration of oligonucleotides in D_2O solution was typically 75-100 µM.

Result and discussion

In Fig. 2(a), we plot a typical IRAS spectrum collected for a mixed solution of oligonucleotides L and R. For comparison is shown a computed spectrum (thin curve), indicated by (L)+(R) in Fig. 2(a), that was obtained by addition of the two individual spectra of oligonucleotides L and R (simulated spectra of the mixture). We can see that the spectrum of Fig. 2(a) shows notable enhancement in absorbance at 1690, 1670 and 1650 cm^{-1}. We interpret that these spectral changes are due to the formation of hydrogen bonds between the two complementary DNAs, that is, 'hybridization'. Previously, it has been reported [2] that spectral features around 1690 cm^{-1} can be attributed to the C=O stretching modes of Thymine and Guanine residues, and spectral features around 1650 cm^{-1} are due to the ring deformation mode of Thymine residue. We therefore suppose that these vibrational modes were affected by DNA hybridization.

To obtain more details of spectral changes due to DNA hybridization, we perform *ab-initio* calculations using Gaussian'03 (Gaussian Inc.). Figures 3(a) and 3(b) show the calculated spectra of the A-T and G-C pairs, respectively. For comparison are shown the calculated spectra obtained by addition of the spectra of the individual

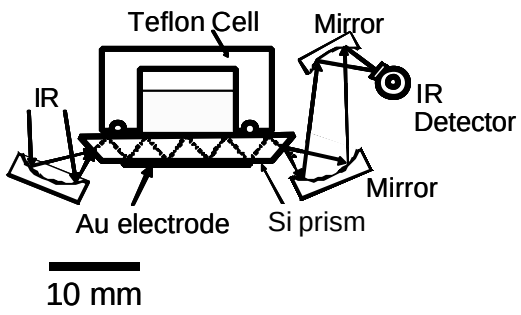

Fig.1: Experimental setup of MIR-IRAS measurement

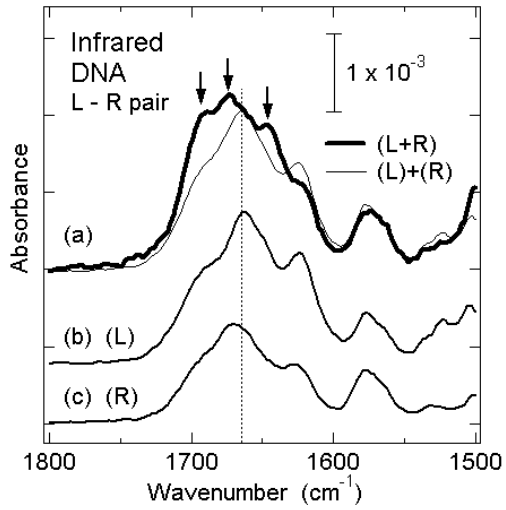

Fig.2: IRAS spectra measured for the complementary pair of oligonucleotides, L - R in a solution of NaCl (100 mM) in D_2O. (a) a mixed solution of L and R, and solutions of (b) L and (c) R.

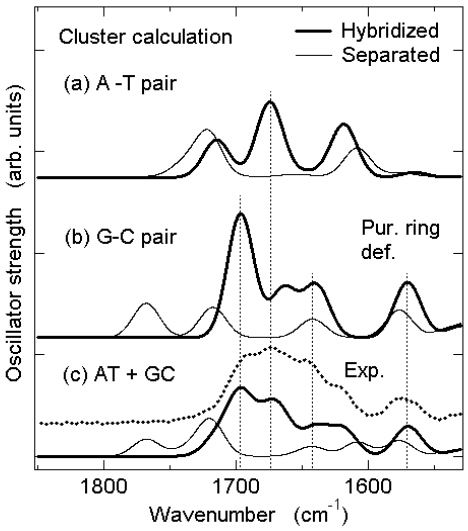

Fig.3: Calculated IRAS spectra of (a) the A-T pair, (b) the G-C pair, and (c) the sum of the two pairs. Thin curves indicate the computed spectra of the separated base pairs. A dotted curve indicates an IRAS spectrum measured for the complementary pair of L - R.

bases (thin curves). The latter spectra indicate the simulation of the separated base pairs. The hydrogen-bonded base pairs exhibit quite different spectral profiles from the corresponding separated base pairs, which suggest that hybridization causes significant spectral modifications in the IRAS spectra of the base pairs. We compare in Fig. 3(c) the calculated spectrum that is the sum of the spectra of Figs. 3(a) and 3(b), with the experimental spectra obtained for the L-R pair. As described in the experimental session, oligonucleotides L and R, have the same number of A-T and G-C pairs, and therefore, the calculated spectrum has been obtained by addition of the calculated spectra of the A-T and G-C pairs with the same weight. Overall agreement in spectral profile between the experiment and calculation can be identified in this hybridized case shown in Fig. 3(c). As described above, we observed that hybridization caused a notable enhancement in absorbance at 1690, 1670 and 1650 cm^{-1}. We can see from a comparison of Figs. 2 and 3 that the observed spectral modifications are due to the conformational changes around the C=O bonds that are involved in hydrogen bonding between the base pairs. Here, it should be pointed out that the hydrogen-bonded base pair is not so much affected by the surrounding water molecules as the single-strand DNA is. This is because the base pairs of the double-stranded DNA are surrounded by the two back bones, and moreover, almost all the C=O bonds are involved in hydrogen bonding. Thus it also suggests that a larger scale models containing the surrounding water molecules are required for detailed analysis of ss-DNA.

Conclusion

We proposed *in-situ* monitoring of DNA hybridization using surface infrared spectroscopy in the geometry of multiple internal reflection geometry. Our experimental results demonstrate that MIR-IRAS is capable of detecting DNA hybridization as spectral changes in D_2O solution. The spectral enhancements at 1690, 1670 and 1650 cm^{-1} due to hybridization are observed. And *ab-initio* calculations are also performed to analysis the details of experimental spectral profiles. The calculation showed that the base pairing due to DNA hybridization has an influence to the vibration modes of C=O bonds. However, more larger scale calculations would be required to determine exactly the origins of the observed spectral changes due to DNA hybridization in solution.

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