Label-free detection and classification of DNA by surface vibration spectroscopy in conjugation with electrophoresis

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Label-free detection and classification of DNA by surface vibration spectroscopy in conjugation with electrophoresis

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We propose a method of *in situ* (*in vitro*) analysis of the chemical bonding state of DNA in aqueous solution by infrared absorption spectroscopy in the multiple internal reflection geometry (MIR-IRAS) in conjugation with electrophoresis. We demonstrate that MIR-IRAS is capable of detecting *in vitro* DNA hybridization. In our method, a Si prism, through which infrared lights penetrate and are also internally reflecting, also serves as the electrode for electrophoresis. We show that with a positive or negative potential being applied to the electrode (prism), DNA molecules are concentrated on or expelled from the electrode, respectively, leading to highly sensitive detection and manipulation of DNA in aqueous solution. © *2005 American Institute of Physics*. [DOI: 10.1063/1.1853529]

In the field of genomics, the focus is now shifting to the gene expression and to the interplay between genes and proteins. To analyze the gene expression and elucidate the DNA-protein or protein-protein interplay, it is quite important to develop a biosensor to analyze and to understand structural and functional relationships of these biomolecules in various clinical states. In general, a biosensor is an electronic device capable of detecting a biological phenomenon by the conversion from biomolecular signals to physical signals. The so-called DNA chip,[1] also known as a "gene-chip," is a typical example of such biosensors. In DNA chips, complementary DNA sequences are detected through DNA hybridization and fluorescence of added chromophores. Various biosensors have so far been proposed and developed for the detection of DNA hybridization. However, as for the chromophores needed for the fluorescence, it is necessary for those biosensors to use labels (biomarkers) in order to distinguish between reacted and nonreacted species.

We propose a label-free method of *in situ* (*in vitro*) analysis of the chemical bonding state of DNA by electrophoresis in conjugation with infrared absorption spectroscopy in the multiple internal reflection geometry (MIR-IRAS).[2,3] The advantages of this method are threefold. First, MIR-IRAS is capable of revealing conformational changes of DNAs through their vibrational fingerprints. Therefore, labeling of biomolecules with fluorescent tags or radioisotopes is not needed for detection and analysis of biomaterials. Second, it is intrinsically sensitive to surface (or interface) vibrations and consequently is suitable for biosensor detection in which only surface or interface phenomena are involved. Moreover, in our method, a Si prism, through which infrared light penetrates, also functions as the electrochemical electrode. By applying a positive or negative potential to the Si electrode (prism), we can manipulate biomolecules in aqueous solutions; for example, negatively charged DNA molecules are moved to the Si electrode under the presence of a positive potential applied to the electrode, leading to highly sensitive detection and classification of DNA.

In the present work, we have selected the chemical system of complementary DNAs as an appropriate template for testing the possible application of our method to biosensors for detecting DNA hybridization. We first collect IRAS spectra for two complementary single-stranded DNAs (ss-DNAs) individually, and then observe infrared spectral changes caused by mixture of the two complementary DNAs in water.

Sample molecules (nucleic acids) used in this study were purchased from Nihon Gene Research Labs Inc., in the purest possible form, and were used without further purification. The sample material was carefully mixed with heavy water (D_2O). The reason we utilized heavy water instead of water (H_2O) as the solvent, is as follows: H_2O has strong scissoring modes around 1650 cm^{-1}, where the bases of DNA have specific vibration modes (C=O stretching and $-NH_2$ scissoring modes) that are quite sensitive to base-pairing. On the other hand, D_2O has no significant vibration modes at this frequency region; it has a scissoring mode at 1230 cm^{-1}. The concentration of DNA in a solution of D_2O was typically 75–100 μM.

Our biosensor (biocell) is almost the same as used in our previous work,[4,5] and is schematically depicted in Fig. 1. Si prisms used for MIR-IRAS measurements were $0.5\times10\times30$ mm^3 with 45° bevels on each of the 10-mm short

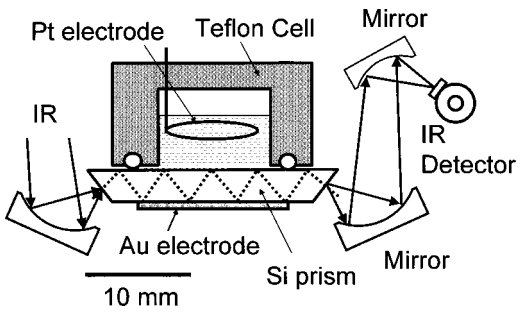

FIG. 1. Biosensor (biocell) for *in vitro* detection of biomolecules.

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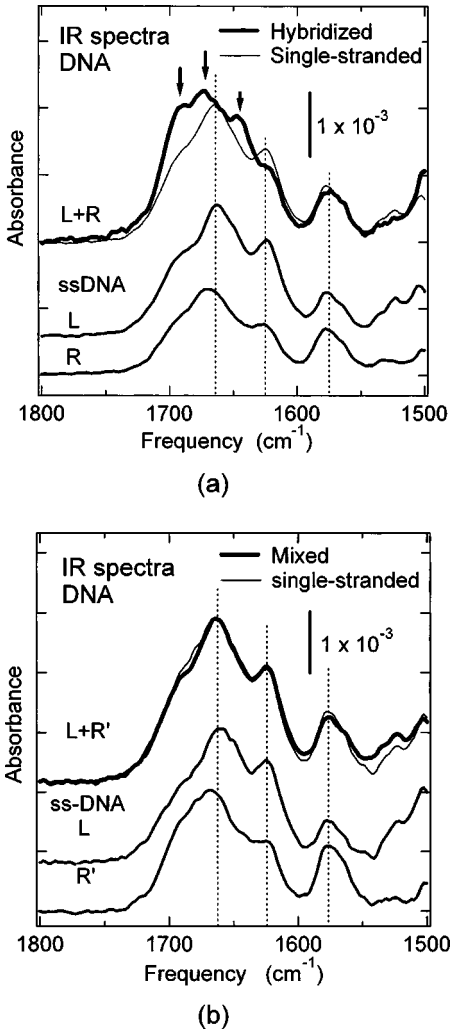

FIG. 2. (a) IRAS spectra of the 30-based, complementary ss-DNAs, and of the hybridized DNA. (b) IRAS spectra of the 30-based, mismatched ss-DNAs, and of the hybridized DNA.

edges. The infrared radiation that exited an interferometer (BOMEM MB-100) was focused at normal incidence onto one of the two bevels of the sample, and penetrated through the wafer, internally reflecting about 50 times, as is shown in Fig. 1. The radiation that exited the sample through the other bevel was focused onto a liquid-N_2-cooled HgCdTe (MCT) detector. The resolution of the interferometer was set at 4 cm^{-1}, which is adequate for defining sets of IR marker absorption bands of the various conformations of DNA molecules.

In order to improve the sensitivity of DNA detection, we have applied a potential to the Si electrode (prism). Thin layers of Au, approximately 10 nm thick, were deposited onto the backside of the Si electrode, as is depicted in Fig. 1. As is well known, DNA has phosphates on its backbone (DNA strand), and consequently is negatively charged. Therefore, we can expect that under the influence of an external electric field, DNA molecules present in D_2O solution move to the Si electrode and are concentrated on the electrode surface; this may be called *electrophoresis*.

We plot in the lower portion of Fig. 2(a) IRAS spectra of two complementary, 30-based ss-DNAs. One of the two DNAs, denoted as "L" in Fig. 2(a), had a nucleotide sequence of 5′-GGAG ACTG TTAT CCGC TCAC AATT CCAC AC-3′, and the other, denoted as "R," was the exact

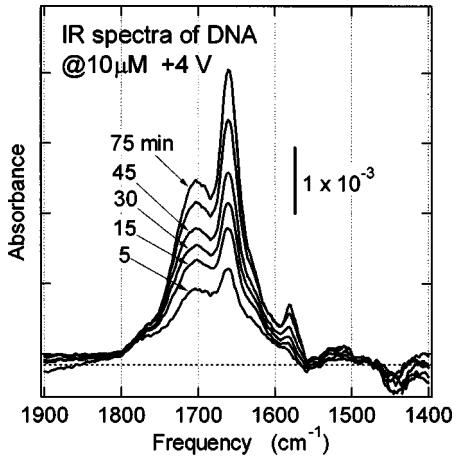

FIG. 3. IRAS spectra of the 30-based ss-DNA (L) under a potential of +4 V applied to the Si electrode.

complement of the former one. These ss-DNAs exhibit similar spectral features in the frequency range of 1500–1750 cm^{-1}. Three broad peaks can be identified at 1580, 1620, and 1680 cm^{-1}. These peaks are mainly due to C=O and C=N stretching vibration modes of the bases in the DNAs.[6,7]

In the upper portion of Fig. 2(a), we plot an IRAS spectrum collected for the DNA solution in which ss-DNA L was mixed with its complementary ss-DNA, R. We carried out several runs of IRAS measurement for this chemical system and obtained similar results for all the runs of IRAS measurement. Therefore, we confirmed the reproducibility of our IRAS measurement. For comparison, we plot in Fig. 2(a) the calculated spectrum that is a simple weighted sum of the spectra collected for the two complementary ss-DNAs individually. This calculated spectrum corresponds to denatured ss-DNAs. It is obvious from Fig. 2(a) that the mixed solution exhibited quite different spectral features from the solution of the ss-DNAs; the absorbance is enhanced around 1700 cm^{-1}. We interpret that these spectral differences are due to DNA hybridization.

Further confirmation of our interpretation is given by the experiments whose results are shown in Fig. 2(b). We immersed ss-DNA L into a DNA solution containing another ss-DNA, denoted as "R′." DNA R′ had the same base composition as DNA R, but had a different nucleotide sequence from DNA R. The base sequence of R′ was 5′-TAGC TGTA CTGG TATG CAAG ACGC TGGT AG-3′. For this pair, DNA hybridization should be definitely forbidden. As is obvious from Fig. 2(b), the spectrum after mixture of the two noncomplementary (mismatched) ss-DNAs was almost the same as the calculated spectrum that is a simple weighted sum of the spectra for the two ss-DNAs (L and R′). This confirms that the spectral differences observed in the spectrum of Fig. 2(a) were specifically induced by DNA hybridization.

Figure 3 shows a series of IRAS spectra of DNA L that have been collected while a positive potential of 4 V was applied to the Si electrode. The figure attached to each spectrum indicates the elapsed time. We can see that the peak intensity increases with time. This clearly indicates that DNA moved to the Si electrode and was gradually condensed in the vicinity of the electrode surface where the intensity of the evanescent field of IR is high. Note that the DNA con-

centration of the solution was 10 μM, which is much lower that that of the DNA solution of Fig. 2; i.e., 100 μM. On the other hand, the dominant peak at 1660 cm^{-1} in the spectra of Fig. 3 is approximately twice as high in intensity than the corresponding peak in the spectra of Fig. 2. Thus, we can determine that our method improves the sensitivity of DNA detection by more than 10 times.

We notice that in the spectra of Fig. 3, a broad peak can be clearly identified at 1700 cm^{-1}. This peak was not observed distinctly in the spectra of Fig. 2. Moreover, the peak observed at 1660 cm^{-1} in the spectra of Fig. 2 was hardly observed in the spectra of Fig. 3. These differences would be due to conformational changes and/or molecular interactions of DNA molecules that are caused by the condensation of the molecules in the vicinity of the electrode surface.

Interestingly, when a negative potential was applied to the electrode, the absorption peaks almost completely vanished, indicating that DNA was expelled from the Si electrode. These observations suggest that by combining MIR-IRAS with electrophoresis, we can not only detect DNA with quite high sensitivity, but also can manipulate DNA in aqueous solution.

In summary, we proposed a label-free method of *in situ* (*in vitro*) detecting and analyzing the chemical bonding state of DNA by a MIR-IRAS biosensor in conjugation with electrophoresis. We demonstrated that DNA hybridization can be detected by analyzing IRAS spectra of DNA in aqueous solution. The present method also allows manipulating DNA by applying a positive or negative potential to the Si prism (Si electrode). This would facilitate a highly sensitive detection as well as classification of DNA. Investigations on the detection of proteins using the present method are now in progress.

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