Fiber-optic Detection of DNA Denaturation for SNP Analysis

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A fiber-optic detection system for SNP analysis has been developed for monitoring the denaturation process of hybridized DNA strands on the end surface of an optical fiber. The probe DNA directly immobilized on the end surface of the fiber was hybridized to target DNA, and the denaturation process was measured. We determined the difference in melting temperature (Tm) between perfectly and SNP-matched probe-target hybrids by derivative analysis of the melting curves.

In the postgenome era, single-nucleotide polymorphism (SNP),[1–3] which is the chiefly investigated subject in genomic variations, participates in various diseases and underlies sensitivity differences to drugs. As expected, there are more than 3 million SNPs coded in the human genome. To identify one's genetic variation type, simple and reliable tools for large-scale SNP analysis are urgently needed. A fiber-optic biosensor enables real-time observation of molecular interactions because the system separates the reaction site from the observation site. The biomolecules immobilized on the surface can remain in reaction solution throughout the measurement. Therefore, the background signal caused by nonspecific binding, which is concomitant with microarray analysis because of the necessary dry-up processing during measuremental procedure, can be eliminated. In this paper, we describe a very simple method for SNP detection with a single optic fiber, one end of which contained immobilized probe DNA molecules, and the hybridization of target DNA molecules was detected by SYBR® Green I. SYBR Green I is commonly used as a double-stranded-DNA-specific indicator to analyze melting curves such as in real-time PCR. The decrease in fluorescence intensity during the denaturation process was measured in a real-time manner. Perfectly matched or SNP-matched samples were identified by their Tm difference.

A schematic of the detection system is shown in Figure 1

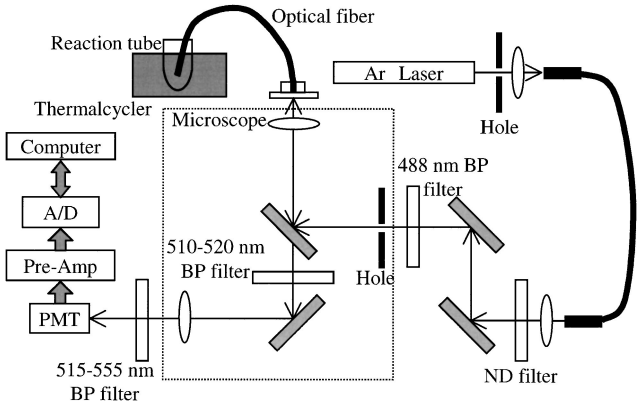

Figure 1. Schematic of the optical setup based on an optical microscope.

and we used aluminum-coated fibers (STVH230M-A, core = 230 μm, Mitsubishi Cable Industries, Ltd.) for its stability in background fluorescence intensity. Aluminum-coat on both ends of the fiber was etched and they were gently cleaved with a sonication cleaver to expose the smooth core surface. One end of a fiber was then used to immobilize probe DNA molecules (reaction end) and the other end was used to introduce the pump beam (incidence end) for the excitation of fluorescence dye on the reaction end. Cleaned reaction end was silanized with 3-aminopropyltriethoxysilane[4] and then probe DNA (5′-AGC GGA TAA CAA TTT CAC ACA GGA-3′) with 5′ amino linker was immobilized using bifunctional cross-linker, glutaraldehyde.[5] Unreacted aldehydes were blocked with glycine. First, dilution series of probe DNA labeled with 5′ amino and 3′ FITC were used in order to find the optimum concentration for immobilization. The amount of DNA molecules immobilized on the fiber surface saturated at a concentration of 12.5 μM (data not shown). The hybridization conditions were also optimized for this system. Hybridization was performed by dipping reaction end of probe-immobilized fiber in a hybridization buffer (TE buffer containing 1/10000 diluted SYBR Green I) for 15 min at 45 °C, which contained 10-μM-target DNA molecules with (5′-TCC TGT GTG AAC TTG TTA TCC GCT-3′) or without (the underlined C was replaced with A) the SNP sequence. After hybridization, melting curves were acquired by ramping the temperature from 45 to 90 °C at a rate of 0.08 °C/s in the hybridization buffer while monitoring the change in fluorescence intensity of SYBR Green I (excitation 488 nm, fluorescence 520 nm) from incidence end.

The melting curves of perfectly and SNP-matched hybrid formed on reaction edge were acquired (data not shown). Also, the melting curve background signal was acquired with intact fiber without hybrid on the reaction edge surface for negative control. The rate of gradual and monotonic decrease in the melting curves of perfectly and SNP-matched target hybridized fibers at the initial (45–55 °C) and the final (75–90 °C) stages agree well with the rate of the intact fiber. This constant decrease is attributed to the background photobleaching of SYBR Green I caused by heating and lasing. The most important part of this curve is the rapid decrease in the fluorescence intensity of perfectly and SNP-matched target hybridized fibers occurring at 55–75 °C. At this temperature, hybrids on the reaction end surface melt at the sequence-specific temperature and the analysis of this Tm difference makes a one-base mismatch detectable. The melting temperature becomes clearer by plotting the negative first derivative of the melting curve (Figure 2a). For comparison, melting temperature of the hybrid in a solution was measured (Figure 2b) using our fiber detection system by simply dipping an intact fiber into the hybridization solution, and also the melting temperature of DNA immobilized on a plate was measured (Figure 2c) using commercially available SNP analyzing system, MCA (Thermo BioAnalysis Japan K. K.)[6] We decided the wide peak

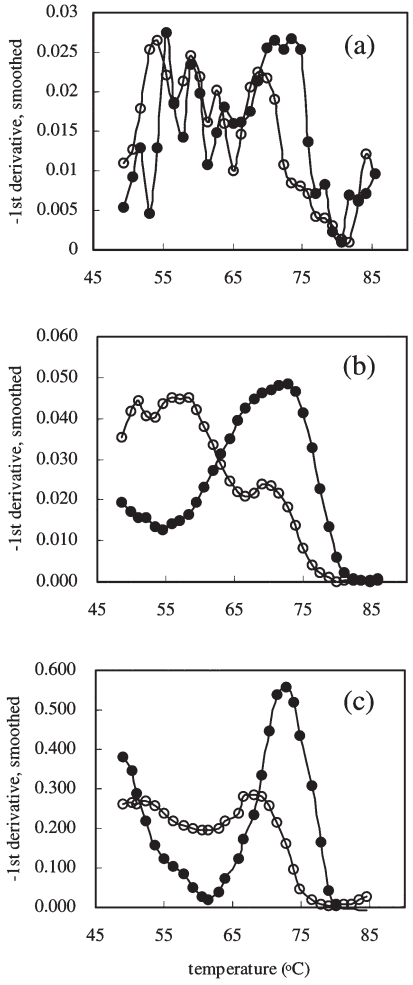

Figure 2. Comparison of smoothed negative first derivative of melting curves: (a) hybrids formed on the end surface of fiber; (b) hybrids in solution detected using an optical fiber; (c) hybrids formed on a plate surface measured with an MCA system. Data points: ● perfectly matched hybrids; ○ SNP-matched hybrid.

as a valid Tm peak. The peak at 73.5 °C of the perfectly matched hybrid and that at 68 °C of the SNP-matched hybrid obtained on the fiber surface agreed well with those obtained using the other two methods. Other peaks at 65 °C or below appeared very similar to the peaks obtained using other methods, particularly to that of the SNP-matched hybrid. It was already reported that the secondary structures of DNA affect the profiles of melting curves, or sometimes induce failures in melting curve analysis.[7] It is reasonable to attribute peaks at temperatures below 65 °C to the secondary structures of DNA molecules. But the fluctuation of melting curve cannot be ignored. This is due to a power loss in laser inducement and signal acquirement and we are now working on an optimization of optical setup in our detection system.

A fiber-optic biosensor using biotin-streptavidin bond has a risk of damage of bonding by heating at 52 °C or above[8] but our present biosensor with covalent bonding is resistant to the temperature ramping (up to 90 °C). It was also demonstrated that probe immobilized fibers could be reused at least five times by washing with ethanol without the background signal increase or hybrid signal decrease (data not shown). There were many studies on real-time monitoring of the hybridization process using fiber-optic sensors. However, in these studies, the base mismatch signal was detected only by the difference in hybrid signal intensity. The hybridization signal intensity depends on the quantity of probe immobilized on a fiber sensor; therefore, there is probability that the absolute fluorescence intensity does not directly reflect the difference in stability between the perfectly and SNP-matched hybrid. SNP detection using physical property such as Tm differences can be a more reliable method with a low probability of misjudgment.

In this report, only single-fiber detection is presented and discussed but we are now attempting to apply this fiber-optic sensor to a multiple detection system by arraying many fibers. The bundle of fibers and the fiber-array scanning detection system for such bundle are being developed. This type of arrayed fiber-optic detection system will be a flexible, large-scale SNP measurement tool. For example, multiple SNP typing of individual patients can be detected by a single assay using SNP-typing probes immobilized on the fiber array; moreover, this assay can be repeated by simply washing the sensors with ethanol. In conclusion, this system is very simple, easy to manipulate, and can be a large-scale measuremental tool using arrayed fibers.

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