Novel Optical Biosensing for Target Oligonucletides by Using Evanescent Wave Technique

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Recently, new transducing methods such as SPR technique and QCM measurement have been well applied to biosensing for target molecules due to those advantageous points such as simple procedure, real time detection, and high sensitivity. In this background, we have paid a lot of attention to the optical biosensing method using evanescent wave as another convenient biosensing technique. In this study, the 21mer of target oligonucleotide as a model of a fragment of the p53 gene, a tumor suppressor gene, was easily detected by fluorescent measurement using evanescent wave excitation. Competitive hybridization reaction of the Cy5-labeled and the non-labeled target oligonucleotides to the complimentary oligonucleotide probe immobilized on the optical wave guide was monitored by real time fluorescence measurement under evanescent wave excitation without washing process. Convenient identification of the single-base mismatch of the 21mer of target oligonucleotide was also examined with 4 kinds of the immobilized probe oligonucleotides.

Detection principle is schematically illustrated in Figure.1. The employed model oligonucleotide corresponds to the codon 245-251 in exon 7 of p53 gene. Perfect match sequence has the base, G at the center of the codon 248 position, a hot spot of p53 gene. Biotin-labeled probe oligonucleotide was immobilized onto the avidin-adsorbed PMMA cuvette. Inner wall of the PMMA cuvette was used as an optical wave guide. Hybridaization reaction upon injection of Cy5-labeled target oligonucleotide was monitored at 37 ^{o}C with the evanescent wave-excited fluorescence detector (Nissui–Pharm. Co. Ltd). PBS solution (pH7.4) was used as a washing buffer if need.

Fluorescence intensity increased rapidly after injection of Cy5-labeled target DNA into the probe DNA-immobilized cuvette. The intensity change indicated that hybridization reaction came to equilibrium within about 15 minutes after injection. And the intensity change was depended on the target DNA concentration. It was demonstrated that the target nucleotide was detected at the concentration range from 10^{-8} to 10^{-7} M. Furthermore, highly specific hybridaization of the perfect match of DNA was confirmed with the 13mer of probe DNA.

Next, simple biosensing of the non-labeled target DNA was examined by evanescent wave-fluorescence measurement of competitive hybridization with Cy5-labeled target DNA to the probe DNA. As the result, Non-labeled target DNA was simply detected by competitive hybridization measurement in the concentration range from 10^{-8} to 5 x 10^{-6} M.

Furthermore, we have examined simultaneous biosensing for multi-target oligonucleotides which were labeled with different dye by evanescent wave absorption spectrometry. The data will be introduced in this presentation.

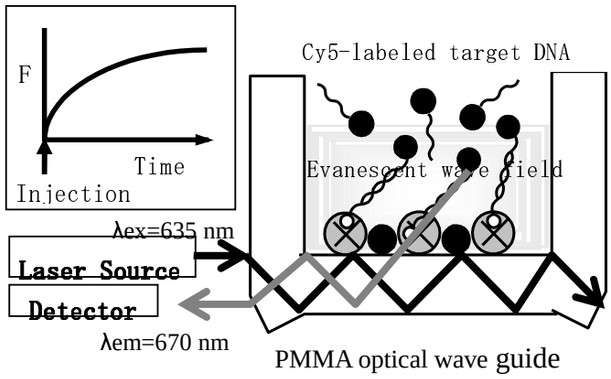

Fig.1 Schematic illustration of the detection principle and the measurement cuvette.