Electrochemical Gene Detection Using Microelectrode Array on a DNA Chip

Yong-Sung Choi*, Young-Soo Kwon and Dae-Hee Park*****

Abstract - In this study, a DNA chip with a microelectrode array was fabricated using microfabrication technology. Several probe DNAs consisting of mercaptohexyl moiety at their 5' end were immobilized on the gold electrodes by a DNA arrayer. Then target DNAs were hybridized and reacted with Hoechst 33258, which is a DNA minor groove binder and electrochemically active dye. Linear sweep voltammetry or cyclic voltammetry showed a difference between target DNA and control DNA in the anodic peak current values. It was derived from Hoechst 33258 and concentrated at the electrode surface through association with the formed hybrid. This suggested that this DNA chip could recognize the sequence specific genes.

Keywords: DNA minor groove binder, Hoechst 33258, Linear sweep voltammetry Mercaptohexyl moiety, Microfabrication technology,

1. Introduction

The detection of a sequence-specific gene is of great significance in the biomedical field. A biosensor using DNA as the biosensing element is called a DNA sensor, and an integrated DNA sensor is particularly referred to as a DNA chip or DNA microarray. DNA microarray technology using photolithography or stamping methods enables simultaneous analysis of thousands of sequences of DNA for genetic and genomic diagnostics and gene expression monitoring. Affymetrix [1-3] has developed GeneChip® using a photolithography technique. Brown [4-6] has developed a DNA microarray using a DNA arrayer, which is an automated instrument employed to fabricate DNA chips.

Conventional DNA chip systems employ confocal fluorescence detection for highly sensitive imaging with high resolution. They detect more than ten thousand of unique oligonucleotides in several square centimeters. Target DNAs are labeled with fluorescent dyes and hybridized with a complimental probe on the chip. DNA chips and microarray scanners using fluorescent detection are very expensive so they are used only at research institutes or large hospitals.

Electrochemical detection has an advantage in reducing the size of the total detection system. Microelectrode array on a DNA chip is superior to fluorescent detection as regards to the cost, portability and convenience. Recently, some electrochemical DNA sensors [7-9] have been developed using electrochemically active DNA intercalators (metal coordination complexes, antibiotics, etc.). Thorp [8, 9] used Ru $(bpy)_3$2+ (bpy=2,2'-bipyridine) as a detection marker for hybridization reaction and detected a single base pair mismatch.

Hashimoto [10, 11] has reported sequence-specific gene detection using a gold electrode modified with probe DNA. He used 2'-(4-hydroxyphenyl)-5- (4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole trihydrochloride (Hoechst 33258) as a detection marker for hybridization reaction, which is a DNA minor groove binder [12] and electrochemically active dye. Hoechst 33258 is concentrated at the electrode surface through association with formed hybrid and irreversibly oxidized on the bare gold electrodes at about 550 mV. In this study, these electrochemical signals were measured to detect specific viral DNAs, for example, human immunodeficiency virus (HIV) or hepatitis C virus (HCV).

However, no integrated multichannel electrochemical DNA sensor has been developed. Microfabrication technology [13-16] has already been established and enables the advancement of miniaturization and mass production of DNA chips. In this study, microfabrication technology was utilized to fabricate an integrated microelectrode array. The main purpose of this study is to develop a device for multichannel electrochemical gene detection. In this paper, we described the results of electrochemical gene detection using a microelectrode array on a DNA chip to develop a clinical gene diagnostic system.

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2. Experimental

2.1 Materials and Instrumentation

Probe DNA (28-mer deoxyoligonucleotide) containing mercaptohexyl group at the 5'-phospate end, and target DNA, which was complementary to the probe, were synthesized and purified. A DNA arrayer motion system was used to immobilize probe DNAs.

HIV-1 SK38; 5'-ATTTCTCCTACTgggATAggTggATT AT-3' and SK39; 5'-gCATTCTggACATAAgACAAggAC CAAA-3' are probe DNA. Target DNA is 5'-CCCCCCCT CCCgggAgAgCCATAgTTggT-3' and 1 base mismatched DNA is 5'-CCCCCCCTCCCgggAAAgCC ATAgTTggT-3'.

The electrochemical measurements were carried out using an electrochemical analyzer manufactured by Bioanalytical Systems, Model BAS-50W and a computer system with data storage. Voltammetric experiments were performed in a Teflon cell including a platinum wire as counter electrode and Ag/AgCl as reference.

2.2 Fabrication of Microelectrode Array on a DNA Chip

Fig. 1 shows the fabrication process of a microelectrode array. A 200 nm gold layer was deposited over a 20nm chromium adhesion layer on a glass chip by vacuum evaporation. Next, the chip was spin-coated with photoresist and was irradiated with UV light. Each metal layer was etched to form electrodes, lead wires, and their connections. The lead wires were photolithographically covered with photoresist for insulation. Thirty-two individually addressable gold electrodes (electrode area: $200\times200\mu m^2$) were arranged on the chip. Each microelectrode was connected to an external potentiostat by insulated gold track. Probe DNAs consisting of mercaptohexyl moiety at their 5'-end were spotted on the gold electrode using micropipette or a DNA arrayer and allowed to react at 10°C for 12 h utilizing the affinity between gold and sulfur. The immobilized probe DNA on the gold electrodes was confirmed by cyclic voltammetry in 5mM ferricyanide/ ferrocyanide solution at 100mV/s.

2.3 Electrochemical Gene Detection with the DNA Chip

Target DNA (complementary) or control DNA was hybridized at 37°C for 1 hour and allowed to react with 100μM Hoechst 33258 for 10 min under a dark condition. After washing the electrodes, electrochemical signals derived from Hoechst 33258 were measured by Linear Sweep Voltammetry (LSV) or Differential Pulse Voltammetry (DPV).

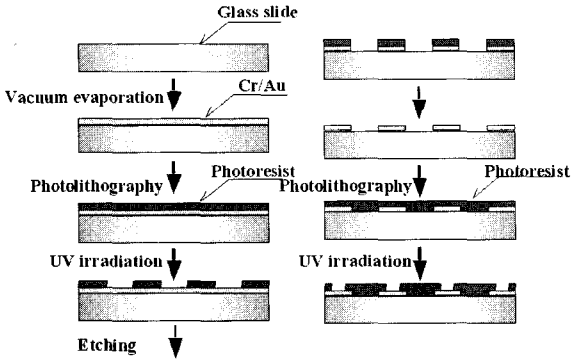

Fig. 1 Fabrication process of microelectrode array.

3. Results and Discussion

Fig. 2 shows cyclic voltammograms (CV) in 5mM ferricyanide/ferrocyanide solution with a bare electrode and a probe-modified electrode (ssDNA-electrode). The peak currents decreased and the separation of peak potential increased when the ssDNA-electrode was used compared with that of the bare electrode. This result shows that the DNA probe is immobilized on the gold electrode through the mercaptohexyl group at the 5' end.

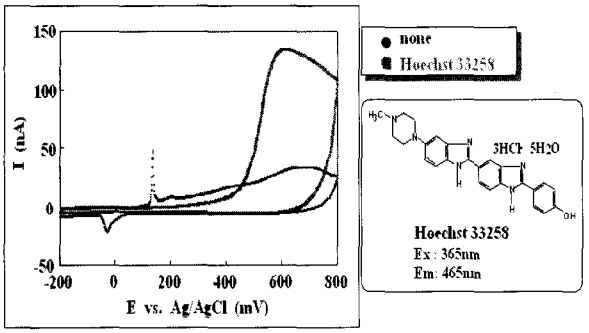

Fig. 2 Cyclic voltammograms of Hoechst 33258 at microelectrode.

Fig. 3 shows linear sweep voltammograms of Hoechst 33258 on probe-modified electrodes reacted with 10nM target DNA and control DNA at 100mV/s. Voltammetric experiments were carried out in the phosphate buffer. When the probe-modified electrodes were reacted with 10nM target DNA (HIV SK38), the anodic peak current values were about 60nA. On the other hand, the anodic peak current values hardly increased when the electrodes were reacted with 10nM control DNA (HIV SK39). It is considered that the increased current value is derived from Hoechst 33258 concentrated on the electrode surface due to hybridization. These results suggest that the microelectrode array specifically detected target DNA.

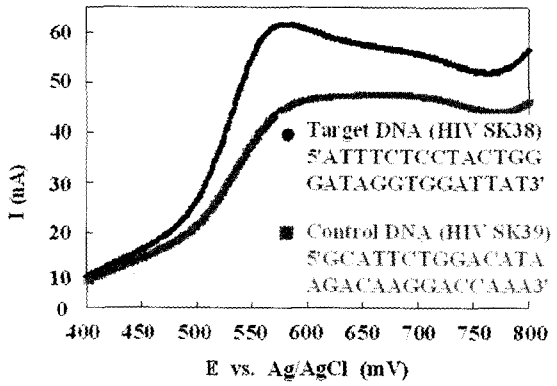

Fig. 3 Linear sweep voltammogram of Hoechst 33258 after hybridization with target DNA and mismatched DNA.

Fig. 4 shows results of detection of a single base pair mismatch. When 100nM target DNA or single base mismatched DNA was detected using differential pulse voltammetry, there was a difference in the anodic peak current values. However, almost no difference in the anodic peak current values was observed for DNA of less than 10nM. Thus improvement of the sensitivity is necessary.

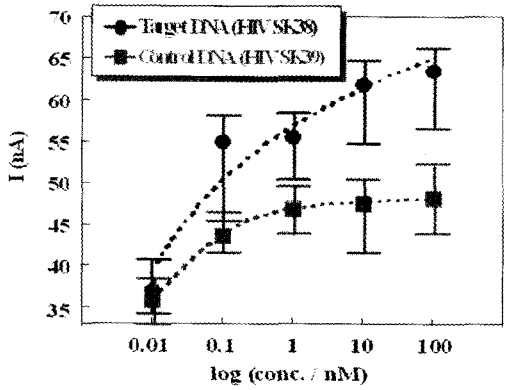

Fig. 4 Calibration curves for concentration of target DNA and control DNA.

Furthermore, the various concentrations of DNA were applied to the measurement. There was a difference between the current values of the target DNA and control DNA ranging from 0.1 to 100nM. The result suggests the DNA microarray can detect the target DNA almost quantitatively. However, the detection sensitivity was too low to detect PCR products. It is also essential to improve the sensitivity.

4. Conclusions

In this study, an integrated microelectrode array was fabricated on a glass slide using microfabrication technology. Probe DNAs consisting of mercaptohexyl moiety at their 5'-end were spotted on the gold electrode using micropipette or a DNA arrayer utilizing the affinity between gold and sulfur. Cyclic voltammetry in 5mM ferricyanide/ ferrocyanide solution at 100mV/s confirmed the immobilization of probe DNA on the gold electrodes.

When several DNAs were detected electrochemically, there was a difference between the target DNA and control DNA in the anodic peak current values. It was derived from specific binding of Hoechst 33258 to the double stranded DNA due to hybridization of the target DNA. However, the detection sensitivity was too low for the use of the genes amplified by PCR, in which case improvement of sensitivity is necessary.

These results suggest that target DNA can be detected specifically by using this microelectrode array. In principle, the method requires no labeling of target DNA. This feature provides simple pretreatment of target DNA. A single channel electrochemical biosensor was already commercialized and its dimension was reduced to palm size. Some electrode arrays of more than several hundred channels were already developed and their size was also miniaturized. This suggested that the multichannel electrochemical DNA microarray is useful to develop a portable device for clinical gene diagnostic systems.

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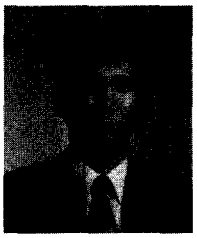

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