Indicator-free DNA Chip Array Using an Electrochemical System

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

Abstract - This research aims to develop a DNA chip array without an indicator. We fabricated a microelectrode array through photolithography technology. Several DNA probes were immobilized on an electrode. Then, target DNA was hybridized and measured electrochemically. Cyclic-voltammograms (CVs) showed a difference between the DNA probe and mismatched DNA in an anodic peak. This indicator-free DNA chip resulted in a sequence-specific detection of the target DNA.

Keywords: DNA probe, Hybridization, Indicator-free DNA chip, Immobilization, Target DNA.

1. Introduction

DNA-based sensors have potential applications that range from genomic sequencing to mutation detection and pathogen identification [1~4]. Conventional DNA chip systems employ confocal fluorescence detection [5~8]. Fluorescent detection DNA chips and microarray scanners are too expensive to use. Electrochemical detection is superior to fluorescent detection as regards to cost, portability and convenience. Recently, electrochemical DNA sensors [9~12] have been developed using electrochemically active intercalators. The above mentioned researches have been performed using fluorescent substances or indicators.

Thus, the purpose of this paper is to develop a DNA chip array without an indicator. We described the results of electrochemical gene detection using a DNA chip array. We fabricated a DNA microarray by microfabrication technology. It is simultaneously able to detect various genes electrochemically after immobilization of various probe DNAs and hybridization of label-free target DNA on the electrodes. Cyclic-voltammograms (CVs) demonstrated a difference between the DNA probe and mismatched DNA against target DNA in the anodic peak. It suggested that this indicator-free DNA chip array could recognize the sequence specific genes.

2. Experimental

The SH-p72 DNA probe, single base mismatched DNAs (SH-m72 and SH-R72) having thiol group at the 5'-end, and target DNAs (p72) were synthesized and purified. A gold layer was deposited over a titanium adhesion layer on a glass by RF sputter. The lead wires were photolithographically covered with photoresist for insulation. Individually addressable gold electrodes were arranged on the substrate. The gold electrodes were reversibly cycled in 10mM H_2SO_4. Then, the electrodes were immersed in the DNA probe solution for 2h at 25°C, and washed with distilled water to remove probes that are not adsorbed. An electrode modified with double-stranded DNA was prepared by the electric field to hybridize the targeted gene at 300mV for 5 sec. The immobilized DNA probe on the gold electrode was confirmed by a voltammetric method using 5mM potassium ferricyanide in a 100mM potassium chloride solution at 100mV/s. The anodic peak current (I_{pa}) was used in the determination of the concentration of the targeted DNA.

3. Results and Discussion

CVs of potassium ferricyanide with the bare gold electrode and the electrode modified with the DNA probe (SH-p72) having the thiol group are shown in Fig. 1 (a) and (b). The peak current decreased by about 59.5% and the peak-to-peak separation (ΔE_p) increased when the DNA probe was used compared with that of the bare electrode (Fig. 1 (a)). This result shows that the DNA probe is immobilized on the gold electrode through the thiol group.The single stranded (ss) DNA-electrodes were reacted with single stranded p72 by applying the electric field in the hybridization buffer. In Fig. 1 (c), the voltammetric data showed that when the ssDNA-electrode was reacted with 1μM p72, the I_{pa} value was decreased by about 72.9%. These results suggest that target DNA p72 can be detected specifically.

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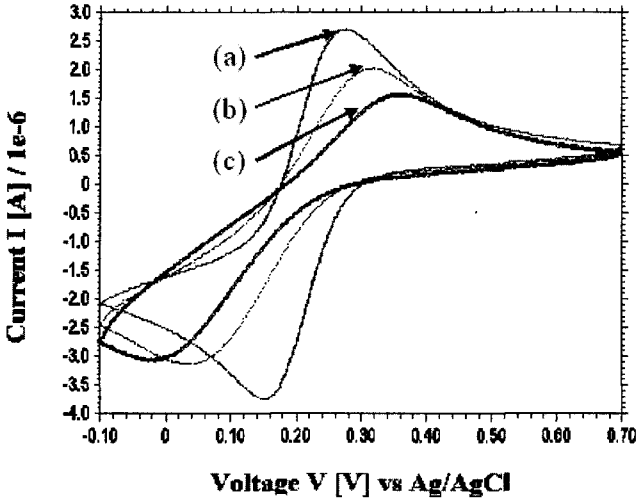

Fig. 1 CVs of 5mM potassium ferricyanide in 100mM potassium chloride at 100mV/s using (a) bare gold electrode (I_1), (b) probe-modified electrode (SH-p72, 5μM; I_2) and (c) after hybridization with target DNA (p72, 1μM; I_3) by the electrical field, where I_1 is the oxidation peak of the bare gold electrode, I_2 is the oxidation peak after immobilization and I_3 is the oxidation peak after hybridization. $(I_2 - I_1)\times100/I_1$ is -59.5% and $(I_3 - I_2)\times100/I_2$ is -72.9%.

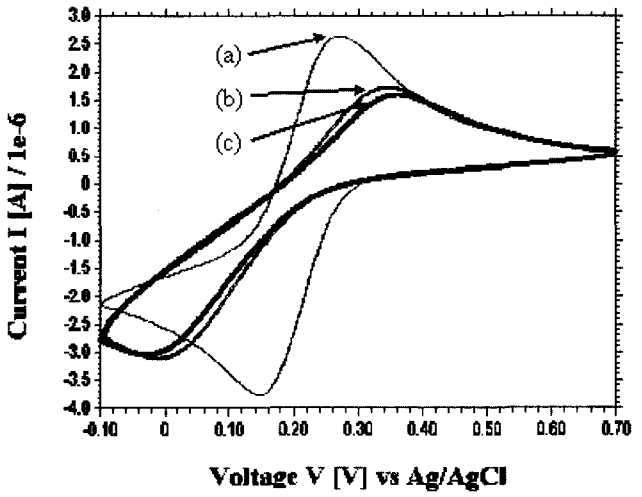

Fig. 2 CVs of 5mM potassium ferricyanide in 100mM potassium chloride using (a) bare gold electrode (I_1), (b) probe-modified electrode (SH-m72, 5μM; I_2) and (c) after hybridization with target DNA (p72, 1μM; I_3), where I_1 is the oxidation peak of the bare gold electrode, I_2 is the oxidation peak after immobilization and I_3 is the oxidation peak after hybridization. $(I_2 - I_1)\times100/I_1$ is -86.4% and $(I_3 - I_2)\times100/I_2$ is -8.9%.

In Fig. 2 (a)/(b) and Fig. 3 (a)/(b), the peak currents decreased by about 86.4% and 21.1% and the ΔE_p increased when the ssDNA-electrodes (SH-m72 and SH-R72) were used in comparison to those of the bare electrodes, respectively. The ssDNAs (SH-m72 and SH-R72) were used for non-specific gene detection, respectively. The ssDNA-electrodes were reacted with single stranded p72 with the same method as in Fig. 1 (c) and CV curves were measured.

After hybridization, the voltammetric data (Fig. 2 (c) and Fig. 3 (c)) showed that when the ssDNA-electrodes were reacted with 1μM p72, the I_{pa} values were almost identical, being decreased by 8.9% and 21.5%, respectively. It could be considered that the ssDNA probes (SH-m72 and SH-R72) almost failed to hybridize with the targeted gene.

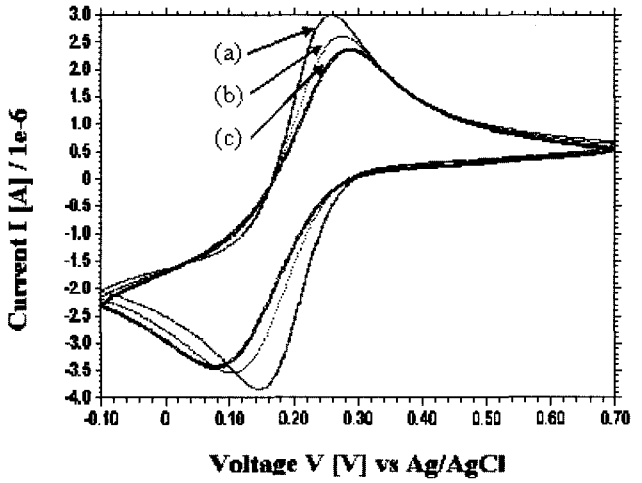

Fig. 3 CVs of 5mM potassium ferricyanide in 100mM potassium chloride using (a) bare gold electrode (I_1), (b) probe-modified electrode (SH-R72, 5μM; I_2) and (c) after hybridization with target DNA (p72, 1μM; I_3), where I_1 is the oxidation peak of the bare gold electrode, I_2 is the oxidation peak after immobilization and I_3 is the oxidation peak after hybridization. $(I_2 - I_1)\times100/I_1$ is -21.1% and $(I_3 - I_2)\times100/I_2$ is -21.5%.

4. Conclusions

In this study, the immobilization of the DNA probe on the gold electrodes was confirmed by CV. After hybridization, when several DNAs were detected without the indicator, there was a difference between the DNA probe and mismatched DNA probe in the anodic peak. This indicator-free DNA chip microarray resulted in the sequence-specific detection of the target DNA. These results suggest that target DNA can be detected specifically without the indicator. This method can be applicable as a

new detection technology to develop various biosensors.

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