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A Study on Gene Detection using Non-labelng DNA

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Abstract

This research aims to develop the multiple channel electrochemical DNA chip using microfabrication technology. At first, we fabricated a high integration type DNA chip array by lithography technology. Several probe DNAs consisting of thiol group at their 5-end were immobilized on the gold electrodes. Then target DNAs were hybridized and reacted. Cyclic voltammetry showed a difference between target DNA and control DNA in the anodic peak current values. Therefore, it is able to detect a plural genes electrochemically after immobilization of a plural probe DNA and hybridization of non-labeling target DNA on the electrodes simultaneously. It suggested that this DNA chip could recognize the sequence specific genes.

Key Words : Multi-channel DNA chip, Microfabrication technology, Photo-lithography technology, Target DNA, Non-labeling

1. INTRODUCTION

The detection of a sequence-specific gene is of great significance in the biomedical field. Biosensor using DNA as the biosensing element is called DNA sensor, and integrated DNA sensor is particularly called 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[1,2] has developed GeneChip using photolithography technique. Brown[3-5] has developed DNA microarray using DNA arrayer which is an automated instrument to fabricate DNA chips.

Conventional DNA chip systems employ confocal fluorescence detection for highly sensitive imaging with high resolution. It detects more than tenthousands of unique oligonucleotide in several square centimeters. Target DNA islabeled with fluorescent dyes and hybridized with complimentaryprobe on the chip. Fluorescent detection style DNA chips and microarray scanners are too expensive to use only a part of research institute or large hospital.

On the other hand, as for electrochemical measurement method, there are the advantage such as the low cost of an analysis device, the simplification of the whole equipment and the analysis time, and the development to the portable DNA chip in comparison with fluorescence measurement method, and the research is carried out[6]. 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)32+ (bpy=2,2′-bipyridine) as a detection marker for hybridization reaction and detected a single base pair mismatch. Also, it is detecting with an indicator-free method, or redox material is modified to probe DNA or target DNA mainly [10]. There are the problems that should be guanine (G) in a base pair, or these methods

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introduce intercalator that reacts to DNA specifically, or redox material is modified to probe or target DNA[11-13].

This research aims to develop DNA chip without indicator. At first, we fabricated DNA chip by lithography technology. Several probe DNA consisting of thiol group at their 5-end were immobilized on the gold electrodes. Then target DNA was hybridized. Cyclic-voltamometry showed a difference between target DNA and control DNA in the anodic peak current. Therefore, it is able to detect a plural genes electrochemically after immobilization of a plural probe DNA and hybridization of non-labeling target DNA on the electrodes simultaneously. It suggested that this DNA chip could recognize the sequence specific genes.

2. EXPERIMENTAL

2.1 Materials and instrumentation

Poly dA (M.W.=16336 g, Tm=68 ℃), poly dT (M.W.=15886 g, Tm=68 ℃), poly dG (M.W.=17136 g, Tm=109 ℃), poly dC (M.W.=15136 g, Tm=109 ℃) probe DNA (50-mer deoxyoligonucleotide acid), p72 probe DNA (5'-HS-AGGCTGCTCCCCCCGTGGCC-3') (M.W.=6012 g, Tm=80.5 ℃) and its mismatched probe DNA (R72; 5'-HS-AGGCTGCTCCCCGC GTGGCC-3') (M.W.=6012 g, Tm=80.5 ℃) having thiol group at 5' end and these target DNA, which was complementary to the probe, were purchased from Nisshinbo Co. after synthesizing. 0.5 μl micropipette was used to immobilize probe DNA.

Acetone and KCl were obtained from Wako Pure Chemical Industries, Ltd.. A positive photoresist of S1818 and its developer (MF319) were obtained from Shipley Co. Inc.. Au plate (99.95 %, t=0.05 mm), Ag (99.99 %, =0.3 mm) wire, Pt (99.98 %, =0.5 mm) wire were purchased from Nilaco Co.. Other chemicals were used without further purification. In clean room process, filtrated water was used.

2.2 Fabrication of DNA chip

The positive photoresist (S1818) was applied to fabricate an electrode on the Au plate using photolithography by a spin coater (1st:500 rpm/3 s, 2nd:3000 rpm/20 s, 3nd:500 rpm/2 s; 1H-D7, MIKASA) and baked at 110 ℃ for 1 min in oven (CLEAN OVEN PUHC-211, ESPEC). A UV light (MA-10, MIKASA) was irradiated to the resist film for 25 sec through a photo-mask. It was developed to form electrode, lead wires, and their connections by dip in developer (MF319) and filtrated water for 30 sec, and drying with N_2 gas. The lead wires were photo-lithographically covered with photoresist for insulation. The electrode was connected to an external potentiostat by insulated gold track.

2.3 Immobilization of probe DNA

0.5 μl of the aqueous buffer solution (100 mM KCl) of probe DNA (50 μM) consisting of thiol group at their 5-end was spotted on the gold electrode using micropipette and allowed to react utilizing the affinity between gold surface and sulfur at 5 ℃ for 24 hr. The immobilized probe DNA on the gold electrodes was confirmed by cyclic-voltammetry in 100 mM KCl solution at 50 mV/s.

2.4 Hybridization of target DNA

Hybridization experiments were performed by exposing the Au plate with the immobilized probe DNA to hybridization buffer solution (100 mM KCl, 5 mM Tris-HCl (pH8.0)) at 5 ℃ for 24 hr and subsequently injecting the target DNA (1aM-50 μM, 0.5 μL). Hybridization was confirmed by cyclic-voltammetry in 100 mM KCl solution at 50 mV/s. Non-complementary DNA binding (1aM-50 μM, 0.5 μL) was also assessed.

2.5 Electrochemical gene detection

The electrochemical measurements were carried out using an electrochemical analyzer manufactured by Bioanalytical Systems, Model BS-1 and a computer system with data storage. Cyclic-voltammetric experiments were carried out in a Teflon cell including Au plate as

working electrode,platinum wire as counter electrode and Ag as reference electrode.

Target DNA (complementary), negative *control* DNA, *control* DNA or *mismatch* DNA was hybridized at 5 ℃ for 24 hr. After washing the electrodes, electrochemical signals derived from the Au electrode were measured by cyclic-voltammetry in 100 mM 20 μl KCl solution at 25 ℃. Cyclic-voltammetry was measured in the range of -400~+700 mV at 50 mV/s. DNA prevents a redox response after immobilization on the Au surface.

3. RESULTS AND DISCUSSION

Figure 1(a) shows cyclic-voltamogram before *immobilization* of Poly dA probe DNA and after hybridization of target DNA in 100 mM KCl solution. In Fig. 1(a) (1) and (2), the redox peak current origin from Au electrode could be observed at +50 mV and 100 mV and the anodic peak current decreased from about 0.33 μA to 0.05 μA when the probe DNA was immobilized on Au electrode compared with that of the bare electrode. It is considered that the effective surface area of the Au electrode decreases by the immobilization of probe DNA and this played a role of insulation layer. This result shows that probe DNA is immobilized on the gold electrode through the thiol group at the 5'-end of probe DNA.Thereafter probe DNA was immobilized on the Au electrode with the same condition. In Fig. 1(a) (3) and (5), the anodic peak current, about 0.05 and 0.048 μA, was almost same compared with that of the probe DNA when negative control DNA and mismatched DNA were hybridized against probe DNA. These results considered that negative control DNA and mismatched DNA were not hybridized with probe DNA. In Fig. 1(a) (4), however, the anodic peak current was decreased to about 0.02 μA when target DNA was hybridized with probe DNA. This result considered that target DNA is hybridized with probe DNA and the effective surface area of Au electrode for redox is more decreased compared with that of probe DNA modified Au electrode. These tendency were also obtained from poly dT probe DNA.

Figure 1(b) shows cyclic-voltamogram before and after immobilization of poly dG probe DNA and hybridization of negative control DNA, mismatched DNA and target DNA. In Fig. 1(b) (1) and (2), the redox peak current of Au electrodecould be observed and the anodic peak current decreased to 0.09 μA when the probe DNA was immobilized on Au electrode. The anodic peak current is higher than that of poly dA because probe DNA is consist of guanine. It is reported that guanine probe have a redox moiety[14]. In Fig. 1(b) (3) and (5), the anodic peak current, about 0.09 and 0.08 μA, was almost same compared with that of the probe DNA when negative control DNA and mismatched DNA were hybridized against probe DNA. In Fig. 1(b) (4), however, the anodic peak current was decreased to about 0.01 μA when target DNA was hybridized with probe DNA. This result considered that target DNA is hybridized with probe.

Figure 1(c) shows cyclic-voltamogram before and after immobilization of poly dC probe DNA and hybridization of negative control DNA, mismatched DNA and target DNA. In Fig. 1(c) (1) and (2), the redox peak current origin from Au electrode could be observed and the anodic peak current decreased to 0.01 μA when the probe DNA was immobilized on Au electrode. In Fig. 1(c) (3), the anodic peak current was increased to about 0.10 μA when target DNA was hybridized with probe DNA. It is considered that target DNA is consist of guanine and anodic peak origin from this. In Fig. 1(c) (4) and (5), however, the anodic peak current, about 0.01 μA, was almost same hybridization of target DNA in KCl solution (sweep rate: 50 mV/s, 25 "). (a) Poly dA. (b) Poly compared with that of the probe DNA when negative control DNA and mismatched DNA were hybridized against probe DNA.

Figure 1(d) shows cyclic-voltamogram after immobilization of p72 and R72 probe DNA and

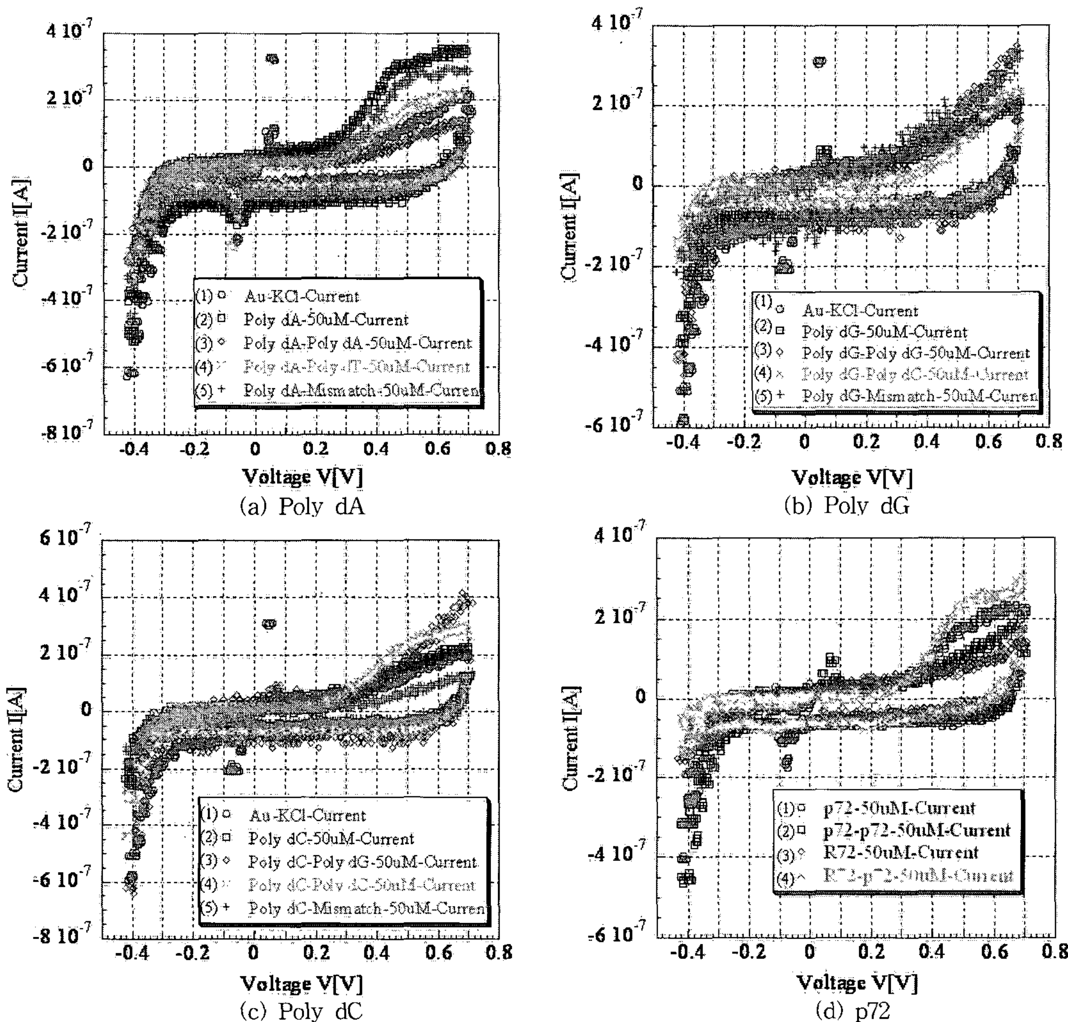

Fig. 1. Cyclic voltamogram before immobilization of probe DNA and after dG. (c) Poly dC. (d) p72.

hybridization of target DNA. In Fig. 1(d) (1), the anodic peak current decreased to 0.06 μA when p72 probe DNA was immobilized on Au electrode. In Fig. 1(d) (2), the anodic peak current was increased to about 0.11 μA when target DNA was hybridized with probe DNA. It is considered that half of target DNA is consist of guanine and anodic peak origin from this. In Fig. 1(d) (3), the anodic peak current decreased to 0.03 μA when R72 probe DNA was immobilized on Au electrode. In Fig. 1(d) (4), however, the anodic peak current, about 0.02 μA, was almost same compared with that of the probe DNA when mismatched DNA were hybridized against probe DNA.

Figure 2 shows concentration dependence of target DNA, negative control DNA and mismatch DNA against probe DNA from 1aM to 50 μM for poly dA, poly dG, poly dC and p72 probe DNA. When target DNA was detected using cyclic-voltammetry, there was a difference and decreased according to the concentration, 1 aM or 1 fM to 50 μM, of target DNA in the anodic peak current. However, almost no difference in the anodic peak current values was observed for negative control DNA and mismatched DNA. The

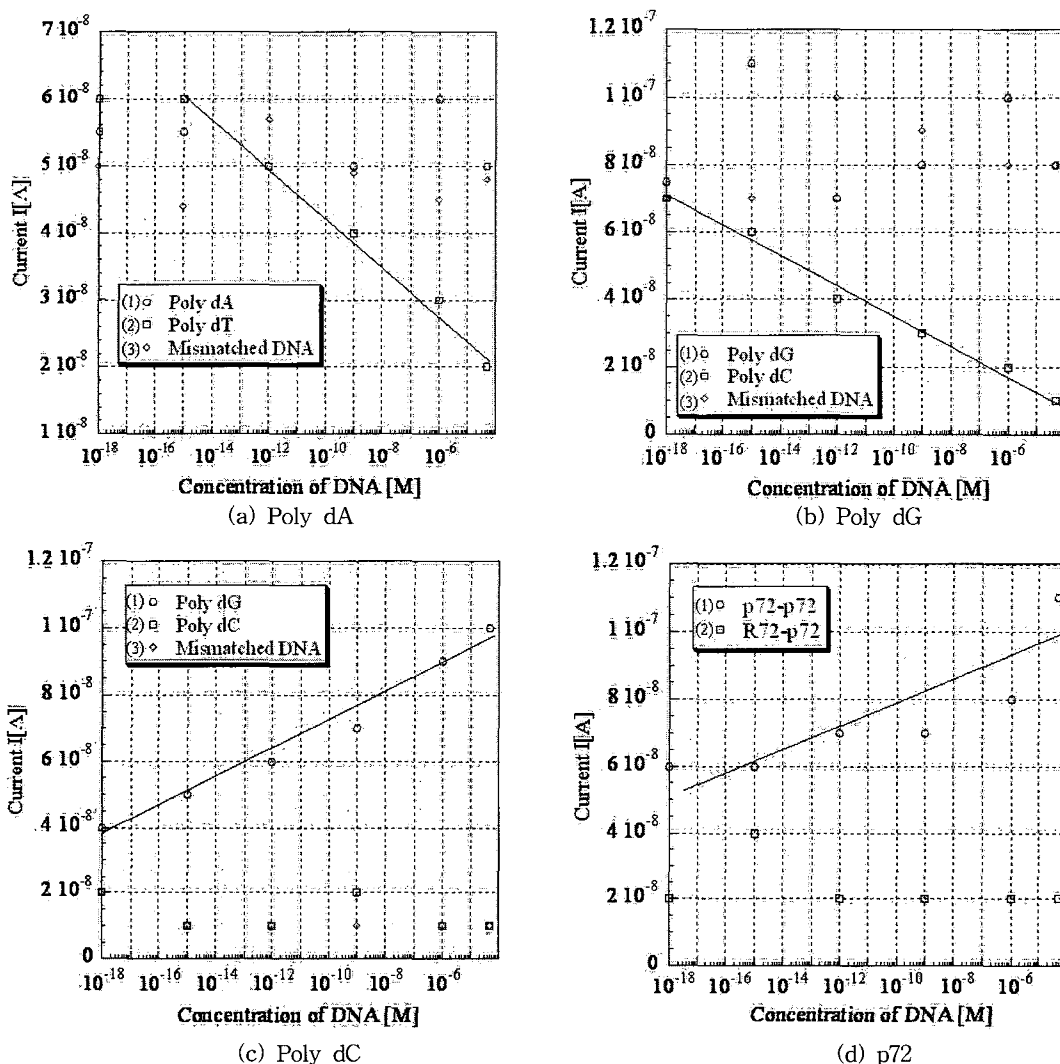

Fig. 2. Concentration dependence of target DNA, negative control DNA and mismatched DNA against probe DNA from 1 aM to 50 μM. (a) Poly dA. (b) Poly dG. (c) Poly dC (d) p72.

result suggests this DNA chip can detect the target DNA almost quantitatively. However, the slope of poly dC and p72 probe DNA against target DNA was reverse due to guanine.

4. CONCLUSION

In this study, DNA chip was fabricated on Au plate using microfabrication technology. Probe DNA consisting of thiol group at their 5-end were spotted on the Auelectrode using micropipette utilizing the affinity between gold and sulfur. Cyclic-voltammetry in 100 mM KCl solution at 50 mV/s confirmed the immobilization of probe DNA on the Au electrodes. When several DNA were detected electrochemically, there was a difference between target DNA and control DNA in the anodic peak current. It was derived from Au due to hybridization of target DNA. The detection sensitivity of gene was fM or aM. These results suggest that target DNA

can be detected specifically by using this non-labeling DNA chip. In principle, the method requires no labeling of target DNA. This feature provides simple pretreatment of target DNA.

REFERENCES

[1] Fodor, S. P. A., Read, J. L., Pirrung, M. C., Stryer, L., Lu, A. T., and Solas, D., "Light-direct, spatially addressable parallel chemical synthesis", Science, Vol. 251, p. 767, 1991.

[2] Fodor, S. P. A., Rava, R. P., Juang, X. C., Pease, A. C., Holmes, C. P., and Adams, C. L., "Multiplexed biochemical assays with biological chips", Nature, Vol. 364, p. 555, 1993.

[3] Schena, M., Shalon, D., Davis, R. W., and Brown, P. O., "Quantitative monitoring of gene expression patterns with a complementary DNA microarray", Science, Vol. 270, p. 467, 1995.

[4] Yoshio O., Masanori K., Kenichi N., Fuyuka O., Hiroyuki F., and Yasuhito E., "Kinetic measurements of DNA hybridization on an oligonucleotide-immobilization 27-MHz quartz crystal microbalance", Anal. Chem., Vol. 70, p. 1288, 1998.

[5] Woolley A. T. and Mathies R. A., "Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips", Pro. Natl. Acad. Sci., USA, Vol. 91, p. 11348, 1994.

[6] Murakami Y., Takeuchi T., Yokoyama K., Tamiya E., and Karube I., "Integration of enzyme-immobilized column with electro-chemical flow cell using micromachining techniques for a glucose detection system", Anal. Chem., Vol. 65, p. 2731, 1993.

[7] Pease A. C., Solas D., Sullivan E. J., Cronin, M. T., Holmes, C. P., and Fodor S. P. A., Pro. Natl. Acad. Sci. USA, Vol. 91, p. 5022, 1994.

[8] Lavigne J. J., Savoy S., Clevenger M. B., Ritchie J. E., McDoniel B., Yoo S.-J., Anslyn E. V., McDevitt J. T., Shear J. B., and Neikirk D., "Solution-based analysis of multiple analytes by a sensor array: toward the development of and a electronic tongue", J. Am. Chem. Soc., Vol. 120, p. 642, 1998.

[9] Adam B. S., Tonya M. H., and Michael J. T., "Electrochemical quantitation of DNA immobilization on gold", Anal. Chem., Vol. 70, p. 4670, 1998.

[10] Sanjay T., Diana P. B., and Fred R. K., "Multicolor molecular beacons for allele discrmination", Nature Biotechnology, Vol. 16, p. 49, 1998.

[11] Marrazza G., Chianella I., and Mascini M., "Disposable DNA electrochemical sensor for hybridization detection", Biosens. Bioelectron., Vol. 14, p. 43, 1999.

[12] Park N. and Hahn J. H., "Electrochemical sensing of DNA hybridization based on duplex-specific charge compensation", Anal. Chem., Vol. 76, p. 900, 2004.

[13] Shana O. K., Elizabeth M. B., Jacqueline K. B., Nicole M. J., and Michael G. H., "Single-base mismatch detection based on charge transduction through DNA", Nucleic Acids Research, Vol. 27, No. 24, p. 4830, 1999.

[14] Mirkin C. A., Letsinger R. L., Mucic R. C., and Storhoff J. J., "A DNA-based method of rationally assembling nanoparticles into macroscopic materials", Nature, Vol. 382, p. 607, 1996.