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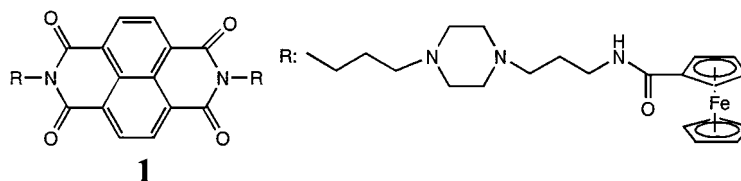
ABSTRACT

An electrochemical DNA sensing technique was developed by using ferrocenyl naphthalene diimide coupled with a probe DNA-immobilized electrode. This technique enabled detection of target DNA quickly and with high sensitivity. Applicability of this technique for single nucleotide polymorphisms (SNPs) analysis was demonstrated by successful analysis of a DNA mismatch on the cancer repression gene p53 with high precision.

INTRODUCTION

The DNA sensing technology based on electrochemical methods is attracting attention as a valuable means of DNA analysis because of its quickness, high sensitivity and low cost (1). Since the DNA microarray is one of the most useful tools for high-throughput analysis of genes in a sample DNA, it is fascinating to develop a new DNA array system based on the electrochemical method (2). For the first time we designed and synthesized an oligonucleotide carrying a ferrocene moiety as an electrochemically active DNA ligand and have been developing an electrochemical detecting method for practical use (3–5). However, one has to

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attach a ferrocene moiety to each oligonucleotide individually. Therefore, we tried to devise a more simple system which can spare this tedious step and can be run with only one electrochemically active ligand. It turned out that ferrocenyl naphthalene diimide coupled with a probe DNA-immobilized electrode is the most successful system, as presented in our recent work (6,7).

We showed previously that ferrocenyl naphthalene diimide **1** binds to double stranded DNA as a threading intercalator and can form a very stable complex with double stranded DNA. This derives from a unique structure of the complex in which the ferrocene moieties of **1** are lying in the major and minor groove of DNA duplex simultaneously and act as an anchor to prevent dissociation of the ligand from double stranded DNA. In other words, the electrochemical signal from the ferrocene part of ferrocenyl naphthalene diimide **1** can be used as an indicator of DNA duplex formation: ferrocenyl naphthalene diimide **1** can be concentrated on the probe DNA-immobilized electrode in proportion to the amount of duplex formation with target DNA. Since ferrocenyl naphthalene diimide **1** can bind to DNA duplex every base pair (5), the complex serves as a pseudo-polyferrocene array and this unique architecture leads to an enhanced electrochemical signal, enabling extremely sensitive detection of target gene. This behavior is also helpful for the mutation analysis (8). We herein show successful analysis of single nucleotide polymorphisms (SNPs) present on cancer repressor gene p53 using this system.

RESULTS AND DISCUSSION

Thiolated 20-meric oligonucleotides were synthesized to analyze the single nucleotide polymorphisms (SNPs) at amino acid residues 72, 175 and 248 on a cancer repression protein p53 (9). The abbreviations and sequences of the oligonucleotides used in this study are given in the caption of Figure together with the electrochemical results. Ten μmol of thiolated oligonucleotide **P72** was immobilized on a gold electrode (2 mm^2) and was allowed to hybridize with 20 μmol of oligonucleotide **P72**- or **R72**- in 5xSSC buffer (83.3 mM sodium citrate buffer at pH 7.0) for 30 min to yield fully matched (**P72/P72**-) and G-G mismatched (**P72/R72**-) duplexes, respectively, on the electrode. Following washing, differential pulse voltammograms (DPV) were measured in an electrolyte containing 1 (0.1 M AcOH-AcOK buffer at pH 5.6 containing 0.1 M KCl and 0.005 mM **1**) at 35°C with a normal three electrode configuration consisting of an Ag/AgCl reference



electrode, a Pt counter electrode, and an indicator (probe DNA electrode). The current peak was observed at 440 mV due to the redox current of the ferrocene parts of **1** concentrated on the electrode. The shape of the peak and the background current were adequate for DPV measurements. The current response at 440 mV corresponding to **1** increased after hybridization with **P72**, demonstrating that **1** is concentrated on the surface of double stranded DNA (**P72/P72**-) more than that of single stranded DNA (**P72** alone). This phenomenon can be used for the monitoring of duplex formation on the electrode, but the currents for the individual electrodes fluctuated as much as 30% on the electrode. Therefore, all data were standardized using the Δi values, defined as $i/i_0 - 1$, where i_0 and i refer to the current before and after hybridization, respectively. The Δi value represents the current from the double stranded DNA formed per probe (single stranded) DNA immobilized on

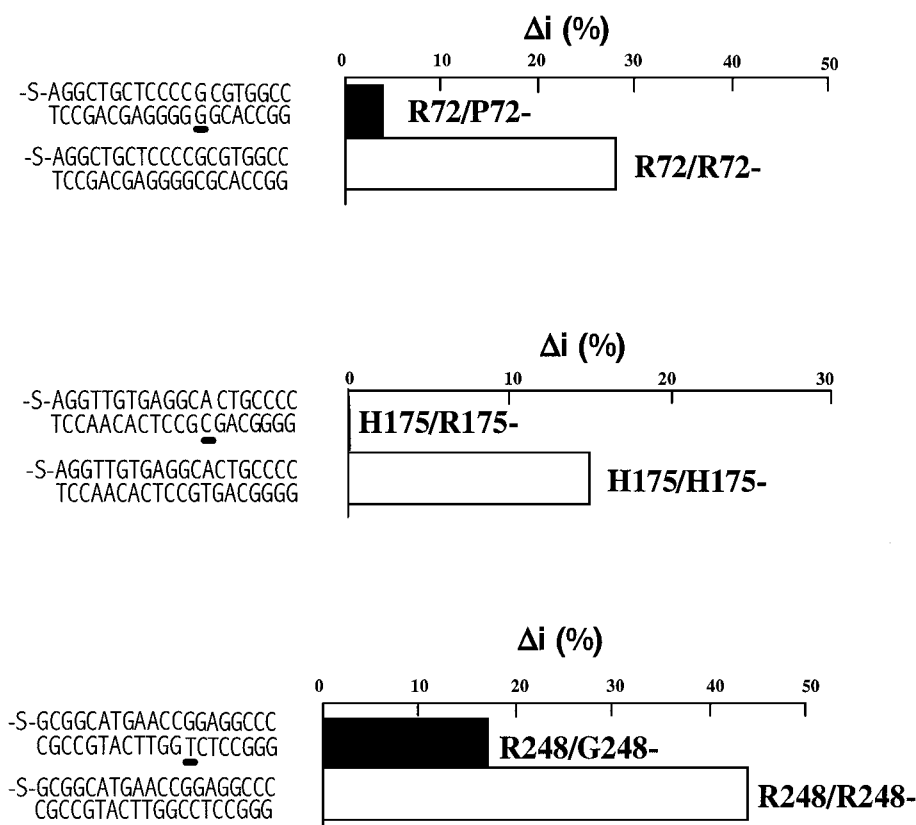


Figure 1. Mismatch detection for the hot spots of p53 gene. Δi values were obtained for the oligonucleotide-immobilized electrodes (**R72**, **H175** and **R248**) and sample oligonucleotides (**R72**, **R72**-, **R175**-, **H175**-, **R248**- and **G248**-). Letters **R**, **H**, **G**, **P** refer to arginine, histidine, glycine, and proline, respectively. The number represents the position of the amino acid in question. Symbol “-” represents the sequence of the non-coding strand DNA, whereas symbols without “-” represent the sequence of the coding strand DNA. The underlined bases represent a mismatch. Solid and open bars represent mismatched and matched combinations, respectively.



the electrode. The Δi value for **P72/P72-** was 42.0%, whereas that for **P72/R72-** was 4.0%. Other SNPs at positions 175 and 248 were also determined analogously and the mismatch was discriminated correctly from the match in all of the runs (Fig. 1). When a PCR product or chromosomal DNA was used, the three types of heterozygote and homozygotes from wild-type and mutants can be discriminated with high precision, too. We are developing a multi-electrode DNA sensor or an electrochemical array (ECA) chip.

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