

METHODS

Highly Sensitive DNA Detection and Point Mutation Identification: An Electrochemical Approach Based on the Combined Use of Ligase and Reverse Molecular Beacon

Zai-Sheng Wu, Jian-Hui Jiang, Guo-Li Shen,* and Ru-Qin Yu

State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, P.R. China

Communicated by Ann-Christine Syvanen

A novel strategy is described for highly sensitive DNA detection and point mutation identification based on the combination of reverse molecular beacon with DNA ligase. A 5'-phosphoryl and 3'-ferrocene terminated DNA sequence is used as detection probe, which may be ligated to capture DNA immobilized on an electrode surface in the presence of a target DNA strand that is complementary to the ends of each DNA, since this allows formation of a nicked, double-stranded DNA. The ligation product may form a hairpin structure after the removal of target DNA. By this method, target DNA can be determined in the range from 3.4×10^{-12} to 1.4×10^{-7} M with a detection limit of 1.0×10^{-12} M. In contrast to existing methods based on the conformation change of redox-labeled oligonucleotides, the proposed strategy offers several substantial advantages: first, the background peak current is eliminated as the ferrocene (Fc)-tagged oligonucleotide probe is specifically ligated to capture DNA; second, a "signal-on" mechanism makes the current intensity increase with increasing target DNA concentration; third, improved current signal is obtained due to the formation of the hairpin structure of ligation products. Additionally, the present system exhibits excellent capability to discriminate mutant target sequences from fully complementary target sequences. *Hum Mutat* 0, 1–8, 2007. © 2007 Wiley-Liss, Inc.

KEY WORDS: point mutation identification; reverse molecular beacon; *E. coli* DNA ligase; AC voltammogram

INTRODUCTION

The detection of DNA hybridization is of significant importance to the basic research of genetic diseases and their medical diagnosis and treatment. Consequently, much effort has been recently devoted to the development of DNA sensing methods [Su et al., 1994; Piuuno et al., 1995; Jordan et al., 1997; Galasso et al., 1998; Kelley et al., 1999; Zhou et al., 2000; Cooper et al., 2001; Weizmann et al., 2001; Park et al., 2002; Peterson et al., 2002; Li et al., 2005]. Among various methods, electrochemical analysis has shown great promise and drawn much attention because of its simplicity, high sensitivity, and compatibility with microfabrication technology [Whittemore et al., 1999; Kertesz et al., 2000; Boon et al., 2000; Alfonta et al., 2001; Ozkan et al., 2002; Kim et al., 2003; Wang and Liu, 2003; Wong and Gooding, 2003; Kerman et al., 2004; Zhang et al., 2004; Liu et al., 2005a; Tansil et al., 2005]. In particular, ferrocene (Fc) moieties have been shown to be extremely useful for electrochemical detection of telomerase activity, DNA hybridization, and protein binding, owing to the versatility of synthesized various Fc derivatives and the excellent reversibility of redox reaction involved, as well as the stability during DNA synthesis [Yu et al., 2001; Wang et al., 2003; Kim et al., 2004; Gibbs et al., 2005; Sato et al., 2005; Le Floch et al., 2006]. Recently, the flexibility of the short DNA, the dynamics of electron transport within DNA monolayer, and

the storage stability of self-assembled monolayer used for fabricating electrochemical DNA (E-DNA) sensors have been explored based on the conformation change of a single-stranded DNA modified with a redox-active moiety [Anne et al., 2003; Anne and Demaille, 2006; Lai et al., 2006]. Furthermore, an electrochemical biosensor for quantitative analysis of DNA sequences has been developed using molecular beacon-like conformation change [Fan et al., 2003]. This method is rapid and simple with a wide achievable dynamic range. The target-induced conformation change has also been characterized using ellipsometry and atomic force microscopy [Immoos et al., 2004a]. A similar DNA detection method has been reported based on the conformational transition of a synthesized Fc-triblock molecule [Immoos et al., 2004b], and encouraging results have been

Received 8 September 2006; accepted revised manuscript 26 December 2006.

*Correspondence to: Guo-Li Shen, Hunan University, State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P.R. China. E-mail: glshen@hnu.cn

Grant sponsor: Science Commission of Hunan Province; Grant sponsor: National Natural Science Foundation of China; Grant numbers: 20435010, 20375012 and 20205005.

DOI 10.1002/humu.20487

Published online in Wiley InterScience (www.interscience.wiley.com).

obtained. Reagentless electrochemical sensors for the detection of thrombin or cocaine based on similar principles have also been fabricated [Xiao et al., 2005; Baker et al., 2006; Radi et al., 2006]. However, the reagentless method for electrochemical DNA detection either works in a “signal-off” mechanism that exhibits a limited signal gain or suffers from the problem of a substantial background peak current. To circumvent these drawbacks and to identify point mutations, a novel strategy has been developed here by combining a DNA ligase with a reverse molecular beacon.

The present approach for the fabrication of DNA biosensor and the electrochemical detection of the target DNA sequence is shown in Figure 1. The detection DNA contains a phosphoryl at the 5' end, a ferrocene tag at the 3' end, and a 6-base sequence close to the 3' end complementary to the 5'-thiolated capture DNA sequence close to the 5' end. The gold electrode is first modified with a mixed self-assembled monolayer of the capture sequence and mercaptoethanol (MCE). After “sandwiching” the target DNA between the capture DNA and the detection DNA, ligation reaction is carried out in the presence of an *Escherichia coli* (*E. coli*) DNA ligase. Then, the target DNA is released from the electrode surface by thermal denaturation. Finally, a molecular beacon-like hairpin structure is formed when the resulting

electrode is incubated in hybridization buffer. As a consequence, much higher response current is obtained than that before thermal dehybridization because the electrochemically active molecule, Fc label, is brought in closer proximity to the electrode surface. A distinctly different peak current is obtained if a mutant target DNA sequence is used for sandwich hybridization. Namely, sensitive and sequence-specific DNA detection can be achieved using the present strategy.

MATERIALS AND METHODS

Materials

Oligonucleotides used were synthesized by Takara Biotechnology Co., Ltd (Dalian, China), and sequences of all oligonucleotides are listed in Table 1. After the labeling of the two amine-terminated DNAs with ferrocenecarboxylic acid (Fc-COOH), different detection DNAs were obtained. All oligonucleotide stock solutions were prepared with 0.3 M NaCl, 10 mM phosphate buffer (pH 7.4) solution (0.3 M PBS) (hybridization buffer).

E. coli DNA ligase was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The ligation buffer was prepared before use by mixing 20 μ L of DNA ligase and 100 μ L of 30 mM Tris-HCl

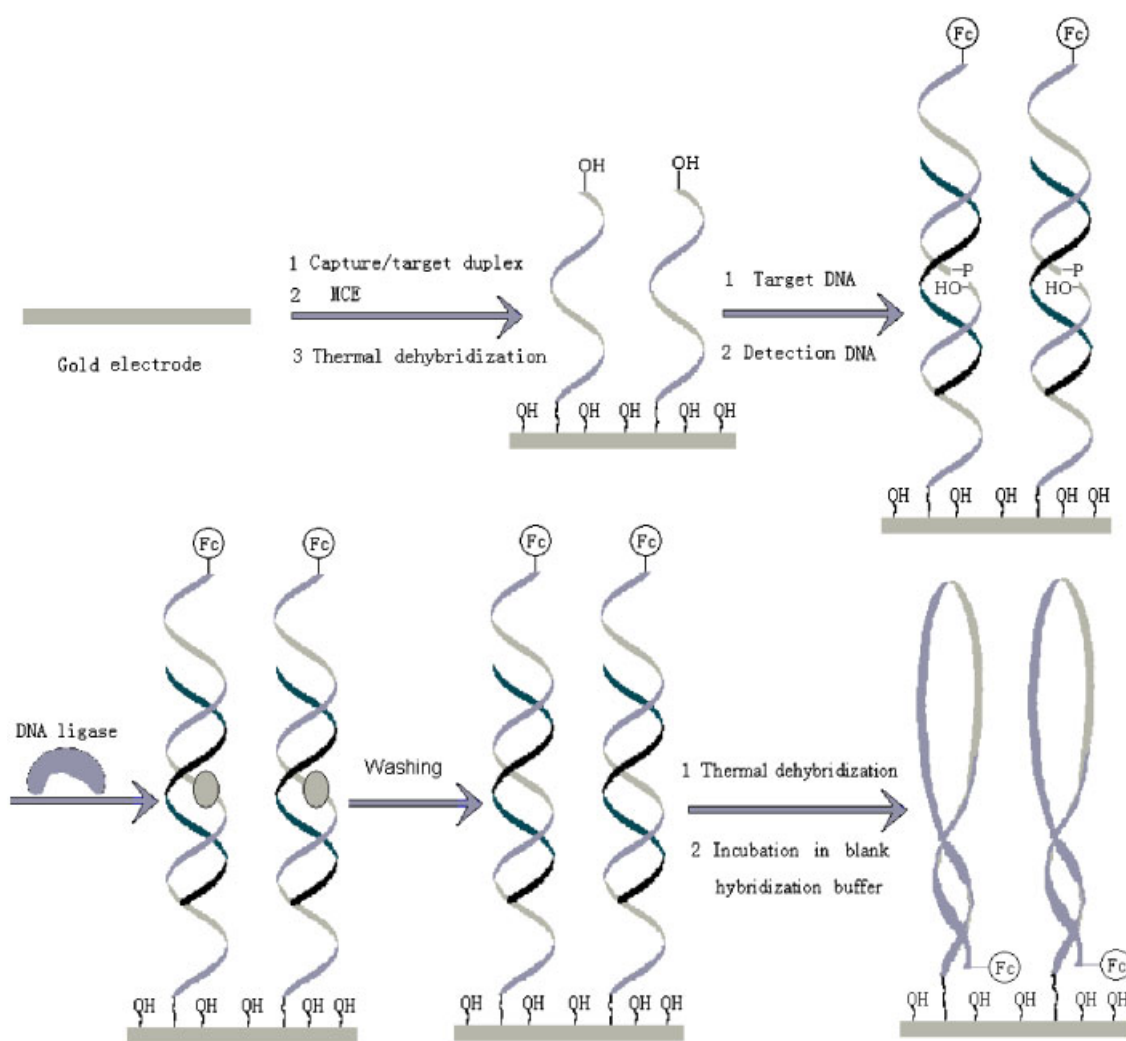


FIGURE 1. Illustration of the strategy for the fabrication of DNA biosensor and the electrochemical detection of target sequence. A capture DNA sequence possessing a terminal thiol is immobilized onto a gold electrode surface. A 5'-phosphoryl and 3'-ferrocene terminated detection sequence is ligated to the sensing interface with enzymatic ligation, resulting in a current response. The hairpin structure obtained holds the Fc label into close proximity to the gold electrode surface, and ensures the efficient redox of the Fc.

TABLE 1. Oligonucleotide Sequences Synthesized in These Experiments

Index	Sequence (5' to 3')	Notes
Capture DNA1 HS	(CH ₂) ₆ -CGTTGATGGTTGGGCCACT	The designed capture sequence
Capture DNA2 HS	(CH ₂) ₆ - <u>TCAACT</u> TGGTTGGGCCACT	A similar sequence to capture DNA1 with italic 6-base mismatches to detection DNAs
Amino-modified DNA1	pTCTCGTGCGCGTTCAACG-(CH ₂) ₃ NH ₂	Precursor of detection DNA1
Amino-modified DNA2	TCTCGTGCGCGTTCAACG-(CH ₂) ₃ NH ₂	Precursor of detection DNA2 without phosphoryl at the 5' end
Target DNA1	CGCGCACGAGA <u>AGTGGCCCAACC</u>	Perfect match
Target DNA2	CGCGCACGAGA GGTGGCCCAACC	Single-base mutation
Target DNA3	CGCGCACGAGA <u>TGTGGCCCAACC</u>	Single-base mutation
Target DNA4	CGCGCACGAGA <u>CGTGGCCCAACC</u>	Single-base mutation
Target DNA5	CGCGCACGAGG <u>AGTGGCCCAACC</u>	Single-base mutation
Target DNA6	CGCGCACGAG <u>T</u> AGTGGCCCAACC	Single-base mutation
Target DNA7	CGCGCACGAG <u>C</u> AGTGGCCCAACC	Single-base mutation
Target DNA8	CGCGCACGAG <u>A</u> AGCGGCCCAACC	Single-base mutation
Target DNA9	CGCGCACGAGA AG <u>T</u> AGCCCAACC	Single-base mutation

(pH 8.0) containing 4 mM MgCl₂, 10 mM (NH₄)₂SO₄, 1.2 mM EDTA, 0.1 mM nicotinamide adenine nucleotide (NAD), and 0.005% bovine serum albumin (BSA). All other chemicals were of analytical grade and were used as received.

Fabrication of DNA Biosensor

The gold electrode consisted of a polycrystalline gold wire sealed in soft glass tube. After being polished and cleaned in an ultrasonic bath, the gold electrode was electrochemically treated in 0.1 M H₂SO₄.

The immobilization experiment was performed using the previous method [Herne et al., 1997] with a minor modification. The cleaned electrode was covered with 20 μL of immobilization buffer (1.0 M KH₂PO₄) containing capture sequence/target sequence duplexes (3.4 μM) for 100 min, and then exposed to an aqueous solution of 1.0 mM MCE for 60 min. The resulting electrode was ready for the electrochemical DNA detection after denaturing the immobilized DNA duplexes by immersing in 0.3 M PBS at 80°C for 10 min.

Measurement Procedure

The capture sequence-modified electrode was held upside down, covered with 20 μL of target sequence solution and incubated for 60 min, followed by washing with water. Then, 20 μL of 3.4 μM detection sequence solution was placed on the electrode surface, and kept the hybridization reaction for 40 min. Subsequently, the ligation reaction was carried out in ligation buffer at 37°C for 40 min after the resulting electrode was cleaned under magnetic stirring. Finally, target sequences were removed from the electrode surface by thermal dehybridization in 1.0 M NaClO₄ solution at 90°C, and the resulting electrode was transferred to another NaClO₄ solution heated to 90°C. The solution was allowed to cool slowly back to room temperature for the formation of hairpin structure.

To evaluate the ability of the developed biosensor to identify a point mutation, a mutant target DNA sequence was used for sandwich hybridization, and detection experiments were carried out as described above.

Electrochemical Measurements

Cyclic voltammetry and alternating current (AC) voltammetry experiments were performed at 37°C using a CHI 760B electrochemical workstation purchased from Shanghai Chenhua Co., Ltd. (Shanghai, China). The auxiliary electrode was a platinum foil, and a saturated calomel electrode (SCE) was used as the reference. All AC voltammograms reported in this study were for the background-subtracted currents.

RESULTS AND DISCUSSION

Experimental Principle

It is well known that, for a reversible reaction, the voltammogram not only includes information about the formal potential of the surface redox reaction but also provides information about the amount of electrochemically-active species fixed on the electrode surface [O'Connor et al., 1999]. Moreover, the distance between the electrode surface and the redox labels determines the electron-transfer efficiency. For nonresonant tunneling of the self-assembled monolayer surface, the rate constant of the electron transfer (k_{et}) decreases exponentially with increasing distance (l) between the redox moiety and the electrode surface, with the decay constants $\beta(= -d \ln[k_{et}]/dl)$ ranging from 0.2 to 1.5 Å⁻¹ [Smalley et al., 2003; Liu et al., 2004]. Therefore, the current response also strongly depends on the molecular length and conformation of redox-labeled molecules. The reported works provide hints for simple systems in which DNA sequences are involved.

Based on the above considerations, the present electrochemical biosensor for the highly sensitive detection of DNA hybridization has been fabricated using the inherent signal transduction mechanism of molecular beacon in combination with the high fidelity of *E. coli* DNA ligase. After the sandwich hybridization and ligation reaction on the capture DNA-modified electrode surface are completed, the response current proportional to the concentration of target DNA is obtained. Then, the system is immersed into hot NaClO₄ solution to remove the target from the electrode surface and incubated in hybridization buffer. Consequentially, a significant signal enhancement is achieved as the formation of molecular beacon-like conformation brings the Fc group closer to the electrode surface.

Immobilization of Capture DNA

Sufficient interstitial space between DNA sequences is necessary to improve hybridization efficiency of surface-confined DNA sequences and to facilitate the subsequent formation of the stem-loop structure. According to the reported literature [Herne et al., 1997; Peterson et al., 2001], the coverage of the thiolated double-stranded DNA on a gold substrate is lower than the density achieved for thiolated single-stranded DNA under the same conditions. Therefore, the DNA duplexes were initially prepared by hybridizing capture sequences with complementary target sequences in order to control surface coverage on the gold electrode. The nitrogen-containing nucleotide side-chains can be adsorbed on the electrode because of interaction with the gold surface, resulting in the nonspecific adsorption of the non-thiol-

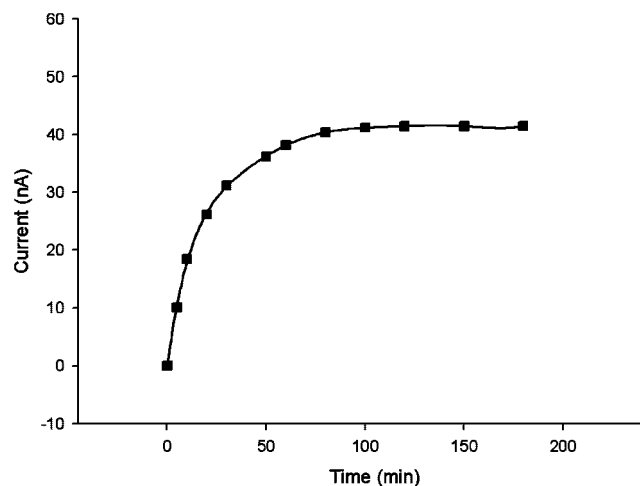


FIGURE 2. Dependence of the background-subtracted peak current on exposure time for the immobilization of the thiolated capture/target duplex prepared by hybridization of capture sequences and complementary target sequences ($3.4 \mu\text{M}$ each) in $1.0 \text{ M KH}_2\text{PO}_4$.

derived DNA [Herne et al., 1997] and the relatively disordered self-assembled monolayer of the thiol-derived DNA and partly preventing subsequent hybridization with target sequences [Levicky et al., 1998]. To improve hybridization activity and to restrict direct access of DNA sequences to the electrode surface [Herne et al., 1997; Wong and Gooding, 2003], the capture DNA/target DNA duplex-modified electrode was immersed into the MCE (served as a spacer and blocking molecule) aqueous solution, because MCE can cure relatively disordered self-assembled monolayer by displacing nonspecifically-adsorbed oligonucleotides [Fan et al., 2003]. After less strongly adsorbed bases were displaced by the thiol groups due to the stronger affinity for gold surface and self-assembled monolayer of thiol-modified DNA reoriented itself [Culha et al., 2003], the majority of dehybridized capture sequences were accessible for DNA hybridization. Moreover, the net negative dipole of the alcohol terminus on the modified electrode surface might repel the negatively charged DNA backbone [Su et al., 2004], leading to the elimination of the nonspecific adsorption of the target sequences and detection sequences. It is important to note that the short carbon chain of MCE does not interfere with the sandwich hybridization and the subsequent self-hybridization of the surface-attached ligation product according to the previous paper [Du et al., 2005].

The influence of the immobilization time of capture sequences on current response was explored. Figure 2 describes the current responses of the different working electrodes used for the target DNA detection. The peak current increased rapidly with the increment of immobilization time and then stabilized after about 100 min. Much longer immobilization time gave essentially the same current response. Therefore, the immobilization time of 100 min was set as the optimal time of the capture sequence immobilization for subsequent experiments.

Electrochemical Characterization of Modified Gold Electrodes

To avoid the instability of ferrocenium (the oxidized form of the ferrocene), 1.0 M NaClO_4 solution was used as the supporting electrolyte when electrochemical behavior of the working electrode was investigated [Fan et al., 2003]. Figure 3 shows cyclic voltammograms of the working electrode used for the

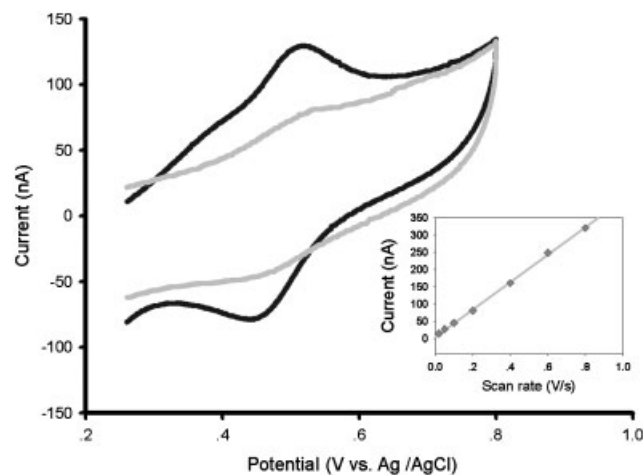


FIGURE 3. Cyclic voltammograms of the ligation product/target sequence complex on the sensing interface before (light gray) and after (dark gray) thermal dehybridization at a scan rate of 100 mV s^{-1} . The concentration of target sequences was $3.4 \times 10^{-8} \text{ M}$, and the electrolyte was 1.0 M NaClO_4 solution. The inset predicts the linear relationship of the peak current against the scan rate in the range of $20\text{--}800 \text{ mV s}^{-1}$, indicating a surface-confined redox reaction only.

detection of target DNA at $3.4 \times 10^{-8} \text{ M}$ before and after thermal dehybridization. A marked change of the redox response was clearly seen after the target sequence was removed from the gold electrode surface. Such a result provided strong evidence that the thermal dehybridization of ligation product/target sequence duplexes and subsequent incubation in hybridization buffer were necessary for efficient electron transfer. The roughly symmetrical shape of CV curve was also observed with an apparent formal potential of 0.478 V (estimated from $E_{1/2} = (E_{red} + E_{ox})/2$, where E_{red} and E_{ox} were the reduction potential and the oxidation potential, respectively) and a relatively low peak separation (about 75 mV) between anodic and cathodic potential, indicating that the redox reaction of ferrocene was basically reversible. The full-width at half-height (fwhh) of either the cathodic or anodic wave is estimated as 118 mV , somewhat larger than the theoretically expected value, 94.2 mV for an ideal Nernstian process of one-electron redox system (Fc/Fc^+) at 37°C , which suggested no significant intermolecular interaction between the immobilized redox moieties [Leavy et al., 1999; Dong et al., 2004]. The dependence of cyclic voltammograms on the scan rates was investigated, and the background-corrected current intensities were plotted against the scan rates. As shown in the inset of Figure 3, the peak current increased linearly with the potential scan rate up to 800 mV s^{-1} , reflecting a surface-confined redox reaction only.

The electrochemical characterization of the DNA-modified electrodes was further performed using AC voltammetry. In each case, the concentration of the complementary target DNA for sandwich hybridization was $3.4 \times 10^{-8} \text{ M}$. The experimental results are shown in Figure 4. Compared with the peak current (Fig. 4; line c) obtained before denaturing, the conformation change of ligation product induced by the hybridization of the stem region resulted in an about eight-fold peak current increase (Fig. 4; line a) when the capture DNA1 and the detection DNA1 were used. In contrast, when the capture DNA2 is substituted for the capture DNA1 for the fabrication of the sensing interface, the current response increased by only five times (Fig. 4; line b) under the same experimental conditions. The difference could presum-

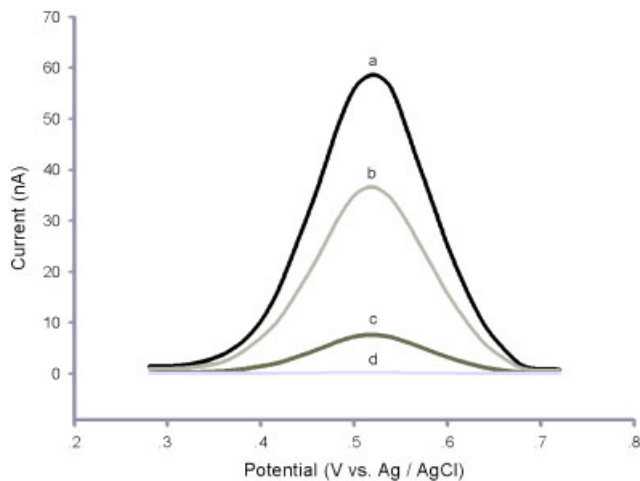


FIGURE 4. Background-subtracted voltammograms for the working electrodes used for DNA detection. **a:** Capture DNA1 was used for the fabrication of sensing interface and detection DNA2 for subsequent DNA detection. **b:** The same as (a) with capture DNA2 to replace capture DNA1. **c:** The same as (a) or (b) without the thermal dehybridization. **d:** The same as (a) with detection DNA2 to replace detection DNA1. Conditions: 1.0 M NaClO₄, amplitude = 50 mV, AC frequency = 1 Hz.

ably be ascribed to the nonexistence of DNA hairpin structure in ligation product that has no complement arm sequences. Although single-stranded DNA was more contractile than a double-stranded DNA, the 3'-Fc-terminated oligonucleotide strand was electrostatically repelled from the sensing interface by the negative dipole of the alcohol moieties and was not efficiently electrochemically accessible [Immoos et al., 2004b; Su et al., 2004]. Apparently, the peak currents decrease in the order from the stem-loop DNA sequences, the stretched single-strand DNA molecules to the target sequence/ligation product duplexes. Based on dependency of the electron transfer rate on upon the distance, this indicates that the distance between electrochemically active labels and the electrode surface might be inclined to decrease as the rigid duplex was denatured to be single-strand DNA, and further decrease after the formation of the hairpin structure. However, if the detection DNA was not phosphorylated at the 5' end, almost no peak current was observed (Fig. 4; line d) as the subsequent ligation reaction could not take place and the detection sequences would be removed from the electrode surface after thermal dehybridization. These observations suggested that the combination of a reverse molecular beacon and a DNA ligase approaches might achieve more sensitive detection of DNA hybridization than either of the two approaches used separately.

Optimization of AC Frequency

AC voltammograms of DNA biosensor used for the detection of target DNA were recorded at various frequencies, and $I_{peak}/I_{background}$ was plotted vs. the logarithm of the frequency, where I_{peak} and $I_{background}$ are absolute peak current and background current, respectively. In general, the AC voltammetric peak current increases with the increasing AC frequency and reaches a maximum at the frequency that keeps pace with the redox reaction rate. For higher AC frequency that is faster than the redox reaction rate, the AC voltammetric peak decreases as the AC frequency increases. As shown in Figure 5, a plateau of the current ratio was seen at low frequency, and a maximum peak current was obtained at 1 Hz; the intermediate part exhibited the gradual diminution of the peak/background current ratio as the

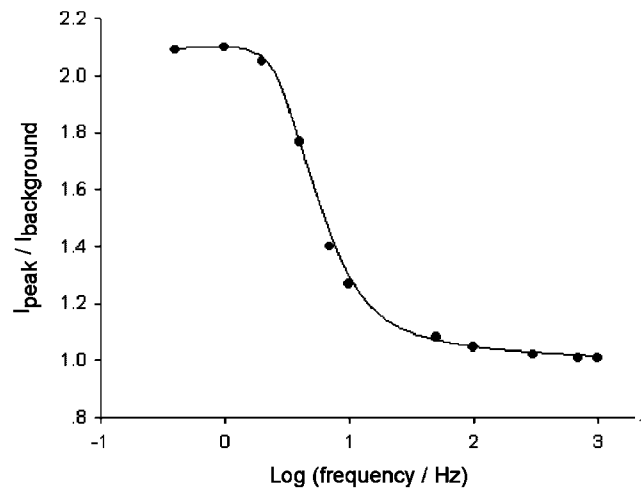


FIGURE 5. The effect of AC frequency on the peak current. The current response is recorded at different working frequency for ligation product with a hairpin structure immobilized on the gold electrode surface. $I_{peak}/I_{background}$ was plotted vs. the logarithm of the frequency, where I_{peak} and $I_{background}$ are absolute peak current and background current, respectively.

working frequency increased; at high frequency, the value of another current ratio tended to 1, which should be attributed to the fact that the redox reaction could not keep pace with the rapidly fluctuating potential at high frequency [Sumner et al., 2001]. Therefore, the AC voltammograms were recorded at the frequency of 1 Hz in subsequent experiments.

Response Characteristics of DNA Sensor

AC voltammetry has proven to be a useful technique for studying redox kinetics in monolayer on electrodes, particularly when the amount of electrochemically active group is small [Creager et al., 1999]. Thus, AC voltammograms were recorded to obtain quantitative information about target sequences. As shown in Figure 6, current intensity increased as the concentration of target DNA increased; no detectable peak current was observed when the primary hybridization was performed in the blank solution. The relationship between peak current of AC voltammogram and the natural logarithm of target DNA concentration is described in the inset of Figure 6. A linear response range covered the concentration range of target DNA sequence between 3.4×10^{-12} and 1.4×10^{-7} M. The regression equation was $Y = 12.37 \log X + 148.7$ with a correlation coefficient of 0.9917, where Y and X were the peak current and the target sequence concentration, respectively. The relative standard deviation was less than 5.0%.

Accurate and sensitive identification of point mutation within the DNA sequence is of central importance to the diagnosis and treatment of genetic diseases. To illustrate the ability of the developed biosensor to discriminate a mutant DNA sequence from a normal DNA sequence, the single-base mutation analysis was performed. Eight cases of single-base mutations in target sequences listed in Table 1 were used for sandwich hybridization instead of fully complementary target sequences. Based on 100% ligation efficiency, defined as that of a fully complementary target sequence, ligation efficiencies of all target sequences involved are shown in Figure 7. No more than 0.6% current responses were obtained for the 10-fold excess of the DNA sequences with point mutations at the nick: compared with other base mismatches, the G:T mismatch induced slightly higher current

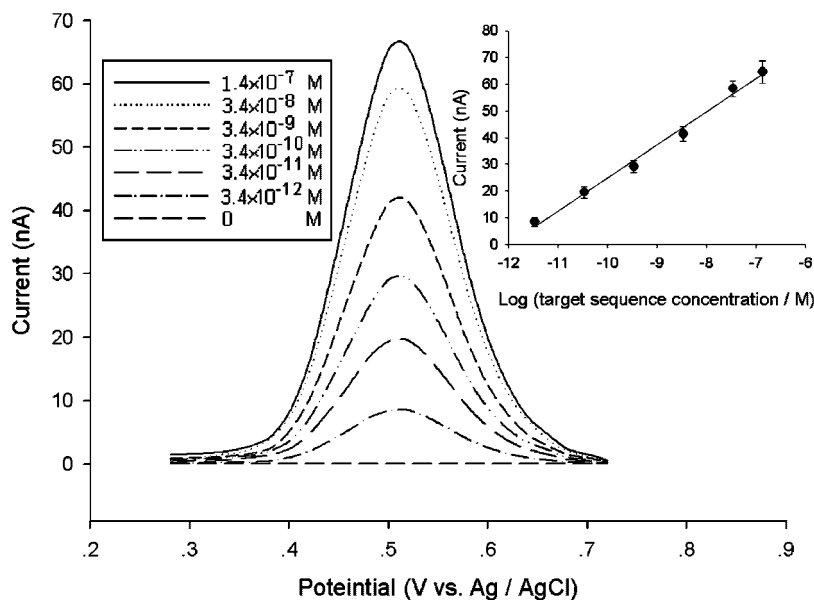


FIGURE 6. AC voltammograms of the working electrodes used for the target sequence detection at different concentrations. Inset: The linear relationship between peak current of AC voltammogram and logarithm of target DNA concentration. The error bars indicate the standard deviation above and below the average of four separate experiments.

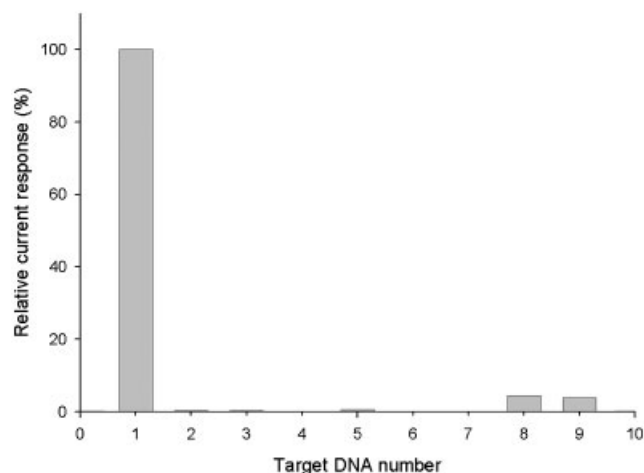


FIGURE 7. Comparison of ligation efficiency of a fully complementary target sequence and different target sequences containing single-base mutations.

response; additionally, *E. coli* DNA ligase exhibited a lower ligation activity when point mutations were on the 3'-side of the nick than on the 5'-side. Single-base mutation at the third or fourth position away from the ligation point only showed less than 5% of the initial ligation efficiency. The high discriminating ability seems to be due to the high fidelity of *E. coli* DNA ligase as well as perturbations in the stability of double-stranded substrates induced by single-base mutations.

High assay sensitivity is important for any viable new DNA biosensor. The present method can achieve a detection limit of 1.0×10^{-12} M (giving a detectable peak current), equating to an absolute detection limit of 20 amol in a 20- μ L droplet of hybridization buffer. Such detectability is 10 times lower than the detection limit in previous works [Fan et al., 2003; Liu et al., 2005b], and the corresponding absolute detection limit is three orders of magnitude lower than the literature value reported by an

enzyme-amplified electrochemical DNA biosensor in an earlier study [Kim et al., 2003]. Such high assay sensitivity is likely due to the elimination of background peak current and the generation of the high peak current originating from the striking decrease of the distance between the redox-active species and the electrode surface through the combined use of a DNA ligase and a reverse molecular beacon approaches.

In summary, in the present study, a novel electrochemical transducer for DNA detection based on the combined use of a DNA ligase and a reverse molecular beacon has been developed and characterized. When compared to the reported method for electrochemical DNA detection based on the conformation change of redox-active molecule-tagged oligonucleotides [Anne et al., 2003; Fan et al., 2003; Immoos et al., 2004b], the present strategy can exhibit several important advantages: the background peak current is eliminated; the peak response is induced by the sequence-specific recognition of target sequences; the height of peak current increases with the increasing target DNA concentration; and higher current response is obtained. Therefore, relatively high sensitivity can be achieved. Additionally, the developed biosensor also exhibits excellent capability to discriminate mutant target sequences from fully complementary target sequences.

REFERENCES

- Alfonta L, Singh AK, Willner I. 2001. Liposomes labeled with biotin and horseradish peroxidase: a probe for the enhanced amplification of antigen-antibody or oligonucleotide-DNA sensing processes by the precipitation of an insoluble product on electrodes. *Anal Chem* 73: 91–102.
- Anne A, Bouchardon A, Moiroux J. 2003. 3'-ferrocene-labeled oligonucleotide chains end-tethered to gold electrode surfaces: novel model systems for exploring flexibility of short DNA using cyclic voltammetry. *J Am Chem Soc* 125:1112–1113.
- Anne A, Demaille C. 2006. Dynamics of electron transport by elastic bending of short DNA duplexes. Experimental study and quantitative modeling of the cyclic voltammetric behavior of 3'-ferrocenyl DNA end-grafted on gold. *J Am Chem Soc* 128:542–557.

- Baker BR, Lai RY, Wood MS, Doctor EH, Heeger AJ, Plaxco KW. 2006. An electronic, aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. *J Am Chem Soc* 128:3138–3139.
- Boon EM, Ceres DM, Drummond TG, Hill MG, Barton JK. 2000. Mutation detection by electrocatalysis at DNA-modified electrodes. *Nat Biotechnol* 18:1096–1100.
- Cooper MA, Dultsev FN, Minson T, Ostanin VP, Abell C, Klenerman D. 2001. Direct and sensitive detection of a human virus by rupture event scanning. *Nat Biotechnol* 19:833–837.
- Creager S, Yu CJ, Bamdad C, O'Connor S, MacLean T, Lam E, Chong Y, Olsen GT, Luo J, Gozin M, Kayyem JF. 1999. Electron transfer at electrodes through conjugated “molecular wire” bridges. *J Am Chem Soc* 121:1059–1064.
- Culha M, Stokes D, Allain LR, Vo-Dinh T. 2003. Surface-enhanced raman scattering substrate based on a self-assembled monolayer for use in gene diagnostics. *Anal Chem* 75:6196–6201.
- Dong T-Y, Chang LS, Tseng IM, Huang SJ. 2004. Electroactive self-assembled biferoceyl alkanethiol monolayers on Au(111) surface and on gold nanoclusters. *Langmuir* 20:4471–4479.
- Du H, Strohsahl CM, Miller BL, Krauss TD. 2005. Sensitivity and specificity of metal surface-immobilized “molecular beacon” biosensors. *J Am Chem Soc* 127:7932–7940.
- Fan CH, Plaxco KW, Heeger AJ. 2003. Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proc Natl Acad Sci USA* 100:9134–9137.
- Galasso K, Livache T, Roget A, Vieil E. 1998. Electrogravimetric detection of DNA hybridization on polypyrrole copolymer. *J Chim Phys* 95:1514–1517.
- Gibbs JM, Park S-J, Anderson DR, Watson KJ, Mirkin CA, Nguyen ST. 2005. Polymer-DNA hybrids as electrochemical probes for the detection of DNA. *J Am Chem Soc* 127:1170–1178.
- Herne TM, Tarlov MJ. 1997. Characterization of DNA probes immobilized on gold surfaces. *J Am Chem Soc* 119:8916–8920.
- Immoos CE, Lee SJ, Grinstaff MW. 2004a. Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *ChemBiochem* 5:1100–1103.
- Immoos CE, Lee SJ, Grinstaff MW. 2004b. DNA-PEG-DNA triblock macromolecules for reagentless DNA detection. *J Am Chem Soc* 126:10814–10815.
- Jordan CE, Frutos AG, Thiel AJ, Corn RM. 1997. Surface plasmon resonance imaging measurements of DNA hybridization adsorption and streptavidin/DNA multilayer formation at chemically modified gold surfaces. *Anal Chem* 69:4939–4947.
- Kelley SO, Boon EM, Barton JK, Jackson NM, Hill MG. 1999. Single-base mismatch detection based on charge transduction through DNA. *Nucleic Acids Res* 27:4830–4837.
- Kerman K, Saito M, Morita Y, Takamura Y, Ozsoz M, Tamiya E. 2004. Electrochemical coding of single-nucleotide polymorphisms by mono-base-modified gold nanoparticles. *Anal Chem* 76:1877–1884.
- Kertesz V, Whittemore NA, Chambers JQ, McKinney MS, Baker DC. 2000. Surface titration of DNA-modified gold electrodes with a thiol-tethered anthraquinone. *J Electroanal Chem* 493:28–36.
- Kim E, Kim K, Yang H, Kim YT, Kwak J. 2003. Enzyme-amplified electrochemical detection of DNA using electrocatalysis of ferrocenyl-tethered dendrimer. *Anal Chem* 75:5665–5672.
- Kim K, Yang H, Park SH, Lee DS, Kim SJ, Lim YT, Tae Y. 2004. Washing-free electrochemical DNA detection using double-stranded probes and competitive hybridization reaction. *Chem Chem Commun* 13:1466–1467.
- Lai RY, Seferos DS, Heeger AJ, Bazan GC, Plaxco KW. 2006. Comparison of the signaling and stability of electrochemical DNA sensors fabricated from 6- or 11-carbon self-assembled monolayers. *Langmuir* 22:10796–10800.
- Le Floch F, Ho HA, Leclerc M. 2006. Label-free electrochemical detection of protein based on a ferrocene-bearing cationic polythiophene and aptamer. *Anal Chem* 78:4727–4731.
- Leavy MC, Bhattacharyya S, Cleland WE Jr, Hussey CL. 1999. Electrochemical and spectroscopic characterization of self-assembled monolayers of unsymmetrical ferrocenyl dialkyl sulfide derivatives on gold. *Langmuir* 15:6582–6586.
- Levicky R, Herne TM, Tarlov MJ, Satija SK. 1998. Using self-assembly to control the structure of DNA monolayers on gold: a neutron reflectivity study. *J Am Chem Soc* 120:9787–9792.
- Li J, Chu X, Liu Y, Jiang JH, He Z, Zhang Z, Shen G, Yu RQ. 2005. A colorimetric method for point mutation detection using high-fidelity DNA ligase. *Nucleic Acids Res* 33:e168.
- Liu B, Bard AJ, Mirkin MV, Creager SE. 2004. Electron Transfer at self-assembled monolayers measured by scanning electrochemical microscopy. *J Am Chem Soc* 126:1485–1492.
- Liu G, Lee TMH, Wang J. 2005a. Nanocrystal-based bioelectronic coding of single nucleotide polymorphisms. *J Am Chem Soc* 127:38–39.
- Liu J, Tian S, Tiefenauer L, Nielsen PE, Knoll W. 2005b. Simultaneously amplified electrochemical and surface plasmon optical detection of DNA hybridization based on ferrocene-streptavidin conjugates. *Anal Chem* 77:2756–2761.
- O'Connor SD, Olsen GT, Creager SE. 1999. A Nernstian electron source model for the AC voltammetric response of a reversible surface redox reaction using large-amplitude AC voltages. *J Electroanal Chem* 466:197–202.
- Ozkan D, Erdem A, Kara P, Kerman K, Meric B, Hassmann J, Ozsoz M. 2002. Allele-specific genotype detection of Factor V Leiden mutation from polymerase chain reaction amplicons based on label-free electrochemical genosensor. *Anal Chem* 74:5931–5936.
- Park SJ, Taton TA, Mirkin CA. 2002. Array-based electrical detection of DNA with nanoparticle probes. *Science* 295:1503–1506.
- Peterson AW, Heaton JR, Geogiadis RM. 2001. The effect of surface probe density on DNA hybridization. *Nucleic Acids Res* 29:5163–5168.
- Peterson AW, Wolf LK, Geogiadis RM. 2002. Hybridization of mismatched or partially matched DNA at surfaces. *J Am Chem Soc* 124:14601–14607.
- Piuono PAE, Krull UJ, Hudson RHE, Damha MJ, Cohen H. 1995. Fiber-optic DNA sensor for fluorometric nucleic acid determination. *Anal Chem* 67:2635–2643.
- Radi A-E, Acero Sanchez JL, Baldrich E, O'Sullivan CK. 2006. Reagentless, reusable, ultrasensitive electrochemical molecular beacon aptasensor. *J Am Chem Soc* 128:117–124.
- Sato S, Kondo H, Nojima T, Takenaka S. 2005. Electrochemical telomerase assay with ferrocenyl naphthalene diimide as a tetraplex DNA-specific binder. *Anal Chem* 77:7304–7309.
- Smalley JF, Finklea HO, Chidsey CED, Linford MR, Creager SE, Ferraris JP, Chalfant K, Zawodzinski T, Feldberg SW, Newton MD. 2003. Heterogeneous electron-transfer kinetics for ruthenium and ferrocene redox moieties through alkanethiol monolayers on gold. *J Am Chem Soc* 125:2004–2013.
- Su H, Kallury KMR, Thompson M. 1994. Interfacial nucleic acid hybridization studied by random primer 32P labeling and liquid-phase acoustic network analysis. *Anal Chem* 66:769–777.
- Su L, Sankar CG, Sen D, Yu H-Z. 2004. Kinetics of ion-exchange binding of redox metal cations to thiolate-DNA monolayers on gold. *Anal Chem* 76:5953–5959.
- Sumner JJ, Creager SE. 2001. Redox kinetics in monolayers on electrodes: electron transfer is sluggish for ferrocene groups buried within the monolayer interior. *J Phys Chem B* 105:8739–8745.
- Tansil NC, Xie H, Xie F, Gao Z. 2005. Direct detection of DNA with an electrocatalytic threading intercalator. *Anal Chem* 77:126–134.
- Wang J, Li J, Baca AJ, Hu J, Zhou F, Yan W, Pang D-W. 2003. Amplified voltammetric detection of DNA hybridization via oxidation of ferrocene caps on gold nanoparticle/streptavidin conjugates. *Anal Chem* 75:3941–3945.
- Wang J, Liu G, Merkoci A. 2003. electrochemical coding technology for simultaneous detection of multiple DNA targets. *J Am Chem Soc* 125:3214–3215.
- Weizmann Y, Patolsky F, Willner I. 2001. Amplified detection of DNA and analysis of single-base mismatches by the catalyzed deposition of gold on Au-nanoparticles. *Analyst* 126:1502–1504.
- Whittemore NA, Mullenix AN, Inamati GB, Manoharan M, Cook PD, Tuinman AA, Baker DC, Chambers JQ. 1999. Synthesis and electro-

- chemistry of anthraquinone-oligodeoxynucleotide conjugates. *Bioconjugate Chem* 10:261–270.
- Wong ELS, Gooding JJ. 2003. Electronic detection of target nucleic acids by a 2,6-disulfonic acid anthraquinone intercalator. *Anal Chem* 75: 3845–3852.
- Xiao Y, Piorek BD, Plaxco KW, Heeger AJ. 2005. A reagentless signal-on architecture for electronic, aptamer-based sensors via target-induced strand displacement. *J Am Chem Soc* 127: 17990–17991.
- Yu CJ, Wan Y, Yowanto H, Li J, Tao C, James MD, Tan CL, Blackburn GF, Meade TJ. 2001. Electronic detection of single-base mismatches in DNA with ferrocene-modified probes. *J Am Chem Soc* 123:11155–11161.
- Zhang Y, Pothukuchy A, Shin W, Kim Y, Heller A. 2004. Detection of $\sim 10^3$ copies of DNA by an electrochemical enzyme-amplified sandwich assay with ambient O_2 as the substrate. *Anal Chem* 76:4093–4097.
- Zhou XC, O'Shea SJ, Li SFY. 2000. Amplified microgravimetric gene sensor using Au nanoparticle modified oligonucleotides. *Chem Commun* 11: 953–954.