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DNA and PNA sensing on mercury and carbon electrodes by using methylene blue as an electrochemical label

Dilsat Ozkan[a], Pinar Kara[a], Kagan Kerman[a], Burcu Meric[a], Arzum Erdem[a], Frantisek Jelen[b], Peter E. Nielsen[c], Mehmet Ozsoz[a,*]

[a] *Department of Analytical Chemistry, Faculty of Pharmacy, Ege University, 35100 Bornova, Izmir, Turkey*
[b] *Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic*
[c] *Center for Biomolecular Recognition, IMBG, Department of Biochemistry B, The Panum Institute, DK 2200 Copenhagen, Denmark*

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Abstract

Described here are the electrochemical parameters for MB on binding to DNA at hanging mercury drop electrode (HMDE), glassy carbon electrode (GCE), and carbon paste electrode (CPE) in the solution and at the electrode surface. MB, which interacts with the immobilized calf thymus DNA, was detected by using single-stranded DNA-modified HMDE or CPE (ssDNA-modified HMDE or CPE), bare HMDE or CPE, and double-stranded DNA-modified HMDE or CPE (dsDNA-modified HMDE or CPE) in combination with adsorptive transfer stripping voltammetry (AdTSV), differential pulse voltammetry (DPV), and alternating current voltammetry (ACV) techniques. The structural conformation of DNA and hybridization between synthetic peptide nucleic acid (PNA) and DNA oligonucleotides were determined by the changes in the voltammetric peak of MB. The PNA and DNA probes were also challenged with excessive and equal amount of noncomplementary DNA and a mixture that contained one-base mismatched and target DNA. The partition coefficient was also obtained from the signal of MB with probe, hybrid, and ssDNA-modified GCEs. The effect of probe, target, and ssDNA concentration upon the MB signal was investigated. These results demonstrated that MB could be used as an effective electroactive hybridization indicator for DNA biosensors. Performance characteristics of the sensor are described, along with future prospects.

Keywords: DNA; Biosensor; Methylene blue; Peptide nucleic acid; Hybridization; Point mutation

1. Introduction

Various tools for DNA-based diagnostics and other areas of biotechnology can be realized based on novel methods for detecting DNA, RNA, and various genetic substructures [1–5]. DNA biosensors based on nucleic acid recognition processes are rapidly being developed towards the goal of rapid and inexpensive testing of genetic and infectious diseases. Electrochemical transducers offer powerful tools for converting the hybridization event into an analytical signal [6]. Nowadays, they are being used in many reports for detecting the DNA hybridization event, due to their high sensitivity, small dimensions, low cost, and compatibility with microfabrication technology [7–9].

The strong affinity of DNA for mercury surfaces has led to the development of adsorptive transfer stripping voltammetric (AdTSV) procedures, which was described by Palecek et al. [10,11]. This procedure is performed with the HMDE immersed in the DNA solution for a short period. The electrode can then be washed and dipped into the blank buffer solution to measure the electrochemical signals of the immobilized DNA at HMDE surface. A small drop of a DNA solution (4–20 μl) is enough for AdTSV procedures, whereas 1–5 ml of DNA is often used in conventional voltammetric analysis. The strong adsorptive immobilization of DNA at HMDE provides an enhancement of the sensitivity by two orders of magnitude compared to pulse polarographic procedures.

Hybridization was detected by redox-active metal complexes that associated selectively and reversibly with double-stranded immobilized DNA [12,13]. Marrazza et al. [14] showed that daunomycin (DM) could be used as an electro-

* Corresponding author. Tel.: +90-232-388-4000x1353; fax: +90-232-388-5258
E-mail address: ozsozs@pharm.ege.edu.tr (M. Ozsoz).

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chemical hybridization indicator for detecting APO E (apolipoprotein E) polymorphisms, using DNA fragments amplified by PCR. Erdem et al. reported that MB [15], epirubicin [16], mitoxantron [17] could be used as redox-active indicators for the electrochemical detection of mismatched bases. Hianik et al. [18] reported that amperometric detection of DNA hybridization on a gold surface depended on the orientation of oligonucleotide chains.

Methylene blue (MB) is an organic dye that belongs to the phenothiazine family. The detection of hybridization was accomplished by using the specific interaction of MB with guanine. The evidence of the direct interaction of MB with guanine bases on the DNA-modified CPEs was also investigated by Yang et al. [19]. Rohs et al. [20] reported a modelling study for MB binding to DNA with alternating guanine–cytosine base sequence. Enescu et al. [21] investigated the conformation of MB–guanine complex by molecular dynamics simulation. The position and orientation of MB–guanine complexes were found to be in three modes: T-shaped, nonstacked, and face to face. Kelley et al. [22] studied the intercalation of MB into the thiol terminated SAM of dsDNA on gold electrode by chronocoulometry, cyclic voltammetry, ellipsometry, and ^{32}P labeling methods. Tani et al. [23] reported that there was a shift in the peak potentials of the square wave voltammetric signals of MB obtained from the thiol-terminated oligonucleotide self-assembly on gold electrode.

Peptide nucleic acid (PNA) is a structural DNA analogue containing an uncharged *N*-(2-aminoethyl) glycine-based pseudopeptide backbone, which has been reported to form Watson–Crick complementary duplexes with DNA [24]. PNA, originally synthesized as a gene-targeting antisense drug, has demonstrated remarkable hybridization properties towards complementary oligonucleotides [25]. Compared to DNA duplexes, PNA hybrids have higher thermal stability and can be formed at low ionic strengths. The neutral peptide-like backbone of PNA provides the basis for the probe to hybridize to target DNA sequences with high affinity and specificity [26,27].

In this paper, we used the HMDE, GCE, and CPE in combination with adsorptive transfer stripping voltammetry (AdTSV), alternating current voltammetry (ACV), and differential pulse voltammetry (DPV) to obtain information about the interaction of MB with DNA. Changes in the MB signal resulting from the indicator interactions with dsDNA, ssDNA, and oligonucleotides have been observed. The features of the method are discussed and compared with those methods reported previously.

2. Experimental

2.1. Apparatus

The cyclic voltammetry (CV) measurements were carried out by using an AUTOLAB PGSTAT 10 electrochemical analysis system and GPES 4.8 software package (Eco Chemie, The Netherlands) in connection with Metrohm VA-Stand 663 (Zurich, Switzerland). HMDE mode was used with the electrode area of 0.40 mm^2. The three-electrode system involved the HMDE, Ag/AgCl/3 M KCl as the reference electrode, and platinum wire as the auxiliary electrode. The following were used as the buffer solution: 0.30 M ammonium formate and 50 mM phosphate buffer solution (pH 6.91).

The alternating current and differential pulse voltammetry (DPV) measurements were done by using an AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.8 software package (Eco Chemie). The three-electrode system consisted of the in-house-made carbon paste electrode (CPE) or a glassy carbon electrode (GCE, BAS, West Lafayette, IN, USA) as the working electrode, the reference electrode (Ag/AgCl, Model RE-1, BAS), and a platinum wire as the auxiliary electrode. The body of CPE was a glass tube (3 mm i.d.) tightly packed with the carbon paste. The electrical contact was provided by a copper wire inserted into the carbon paste. Carbon paste was prepared in the usual way by hand-mixing graphite powder (Fisher) and mineral oil (Acheson 38) in a 70:30 mass ratio. The surface was polished on a weighing paper to a smoothed finish before use. The convective transport was provided by a magnetic stirrer.

2.2. Chemicals

Methylene blue was purchased from La Pine Scientific (USA). The 14-mer PNA oligonucleotide was synthesized at P.E. Nielsen's laboratory, while the 14-mer DNA oligonucleotides were obtained from Genset Oligos (Sydney, Australia). Their base sequences are as below:

DNA target: 5′-GGG GGG CAG AGC AT-3′
PNA and (DNA) probe: H-(5′-) ATG CTC TGC CCC CC (-3′)-LysNH_2
DNA mismatch: 5′-GGG GGG GAG AGC AT-3′
DNA noncomplementary: 5′-TCA AAT CAG GTT GCT TA-3′

Probe is a complementary to 14-base target; mismatch is a mutant of the target with one base changed, as indicated by the underline.

DNA oligonucleotide stock solutions (100 mg/l) were prepared with TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. PNA stock solutions (100 mg/l) were prepared with 50 mM phosphate buffer solution (pH 7.40) and kept frozen. More dilute solutions of DNA and PNA oligonucleotides were prepared with either 0.50 M acetate buffer (pH 4.80) or 20 mM Tris–HCl buffer (pH 7.00), according to the hybridization protocol. Double-stranded calf thymus DNA (dsDNA, activated and lyophilized) and single-stranded calf thymus DNA (ssDNA, activated and lyophilized) were purchased from Sigma. All

DNA stock solutions (100 mg/l) were prepared with TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More dilute solutions of DNA were prepared with 0.50 M acetate buffer (pH 4.80) for DPV and ACV analysis and with 0.3 M ammonium formate, 50 mM phosphate buffer solution (pH 6.91) for CV analysis. The stock solutions of MB (1 mM) were prepared by using distilled water. Other chemicals were of analytical reagent grade. The distilled and deionized water was used in all solutions. Other chemicals were of analytical reagent grade. The in-house-distilled and deionized water was used in all solutions.

2.3. Procedure

2.3.1. Detection of interaction between DNA and MB at HMDE

2.3.1.1. DNA immobilization. The dsDNA- or ssDNA-modified HMDE was prepared by immersing the bare HMDE into a 20-μl drop containing 10 ppm dsDNA or ssDNA in 0.30 M ammonium formate, 50 mM phosphate buffer solution (pH 6.91) for 2 min.

2.3.1.2. MB accumulation. The DNA-modified electrode was subsequently washed with the buffer solution and transferred into the buffer solution, which contained various concentrations of MB. The MB solution was stirred at 200 rpm for 5 min and MB was accumulated onto the dsDNA- or ssDNA-modified HMDE.

2.3.1.3. Voltammetric transduction. The DNA-modified electrode was then subsequently washed with the buffer solution and transferred into the blank buffer solution for the voltammetric measurement. The cyclic voltammograms at HMDE were collected at 500 mV/s scan rate. The cyclic voltammograms at CPE were taken at various scan rates. The raw data were treated using the Savitzky and Golay filter (level 4) of the GPES software. All CV measurements at HMDE or CPE involved a fresh mercury or carbon paste surface. For the measurements performed with the bare HMDE, no DNA was adsorbed onto the electrode surface.

2.3.2. Detection of interaction between DNA and MB at carbon electrodes

DPV measurements required each immobilization/detection cycle at a fresh carbon paste surface. A background scan of buffer alone was collected for each electrode and subtracted from subsequent scans.

2.3.2.1. CPE pretreatment. The CPE was activated by applying 1.70 V for 1 min in 0.05 M phosphate buffer solution with 20 mM NaCl (pH 7.40) without stirring.

2.3.2.2. GCE pretreatment. The GCE was polished with 6- and 1-μm alumina slurries, sonicated in water, and oxidized at 0.50 V for 1 min in 50 mM phosphate buffer solution (pH 7.40). After the oxidation step, GCE was rinsed with water for 10 s.

2.3.2.3. DNA immobilization. dsDNA or ssDNA was immobilized on a pretreated CPE by applying a potential of 0.50 V for 5 min in 10 ppm dsDNA or ssDNA containing 0.50 M acetate buffer solution (pH 4.80) with 20 mM NaCl at 200 rpm stirring. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) with 20 mM NaCl for 10 s. The same procedure above was also performed by using GCE.

2.3.2.4. MB accumulation. The DNA modified electrode was immersed in 20 mM Tris-HCL buffer (pH 7.00) containing 20 μM MB for 5 min at +0.50 V.

2.3.2.5. Voltammetric transduction. The electrode was then transferred into the blank 0.50 M acetate buffer solution (pH 4.80) with 20 mM NaCl for the voltammetric measurement. The differential pulse voltammograms were collected with an amplitude of 10 mV at 20 mV/s scan rate. For the ACV analysis, a frequency of 230 Hz at a scan rate of 10 mV/s was employed with an amplitude of 0.005 V rms. The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a ‘‘peak width’’ of 0.01. For the measurements performed with the bare CPE, no DNA was adsorbed onto the electrode surface.

2.3.3. Gene sequence detection

Each hybridization detection involved the immobilization/detection cycle at a fresh CPE surface.

2.3.3.1. Probe immobilization. The CPE was activated by applying 1.70 V for 1 min in 0.05 M phosphate buffer solution (pH 7.40) without stirring. The pretreated CPE was then washed with water for 10 s and transferred into the probe solution. The probe was subsequently immobilized on CPE by applying a potential of 0.50 V for 5 min in the stirred 10 ppm PNA or DNA probe containing 0.50 M acetate buffer solution with 20 mM NaCl. The electrode was then rinsed with 0.50 M acetate buffer solution for 10 s.

2.3.3.2. Hybridization. The hybridization protocol was performed by dipping the PNA or DNA probe-modified CPE into the 15 ppm target containing 20 mM Tris–HCl buffer solution (pH 7.00) with 20 mM NaCl for 5 min while holding the potential at 0.50 V. The electrode was then rinsed with 20 mM Tris–HCl buffer solution for 10 s and transferred into the blank 20 mM Tris–HCl buffer solution for the measurement. The same procedure as above was repeated by using the mismatch-containing oligonucleotide and noncomplementary sequence instead of the target oligonucleotide which was used instead of the target sequence in the same protocol as explained above.

2.3.3.3. Label binding to the hybrid. MB was accumulated onto the surface hybrid by immersing the electrode into the stirred 20 mM Tris–HCl buffer (pH 7.00) containing 20 μM MB as a hybridization indicator with 20 mM NaCl for 5 min without applying any potential. After accumulation of MB, the electrode was rinsed with 20 mM Tris–HCl buffer (pH 7.00) for 5 s.

2.3.3.4. Voltammetric transduction. The reduction of the accumulated MB was measured by using DPV with an initial potential of 0.20 V in the 20 mM Tris–HCl buffer (pH 7.00) with an amplitude of 10 mV at 20 mV/s scan rate. The raw data were also treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a "peak width" of 0.01.

Repetitive measurements were carried out by renewing the surface and repeating the above assay format.

3. Results and discussion

The ACV signals of MB from the dsDNA-modified, bare, and ssDNA-modified CPEs are displayed in Fig. 1. Due to the interaction of guanine bases with MB at the ssDNA-modified electrode (Fig. 1a), the signal of MB was high in comparison with the one obtained from dsDNA-modified electrode (Fig. 1c). MB displayed a reduction signal at around −0.30 V with CPE at bare CPE (Fig. 1b). The increase of signals at ssDNA-modified electrodes was attributed to the accumulation of the indicator at the electrode surface through interaction with the guanine bases. When the aromatic ring intercalated into dsDNA, the electrochemically active center of MB was enveloped by the bulky DNA molecule and was not available for redox activity, thus Kelley et al. [22] used mediators to accelerate

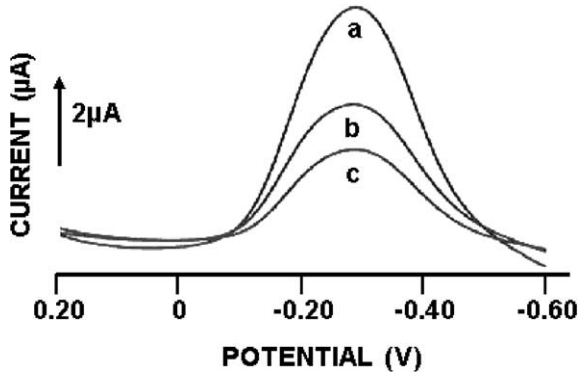

Fig. 1. Alternating current voltammograms of 20 μM MB at (a) ssDNA-modified CPE, (b) bare CPE, and (c) dsDNA-modified CPE. CPE pretreatment: 1 min at 1.70 V in 50 mM phosphate buffer (pH 7.40). DNA immobilization: 5 min at 0.50 V in stirred 10 ppm DNA containing 0.50 M acetate buffer solution (pH 4.80). MB accumulation: 5 min at 0.50 V in 20 mM Tris–HCl buffer (pH 7.00) containing 20 μM MB. Measurement of accumulated MB in 20 mM Tris–HCl buffer (pH 7.00).

this low signal (Fig. 1c). The low signal of MB at bare CPE might be due to the little amount of MB, which might have adsorbed on the CPE surface (Fig. 1b). In spite of these binding and adsorption events, the clearly observed difference in the alternating current responses of MB obtained from dsDNA- and ssDNA-modified CPEs displayed that MB could be utilized as an effective indicator for the detection of hybridization.

Fig. 2 displays the calibration plot of the MB current signals obtained from the AdTSV analysis at HMDE surface, while increasing the concentration of MB. The intercalator accumulated into the base pairs of double helix at the dsDNA-modified HMDE and gave the lowest response at each concentration level (Fig. 2c). The lack of the double-helix form of ssDNA resulted in the high MB signals from the ssDNA-modified HMDE (Fig. 2a). The low signal of MB at bare HMDE might have caused from the adsorbed MB on the HMDE surface (Fig. 1b). Data obtained from all of the modified electrodes during the AdTSV analysis showed saturation behavior with peak currents tending to plateau at concentrations above 100 nM MB.

The calibration plot of the signals of MB adsorbed onto the HMDE surface from its mixture with dsDNA and ssDNA is shown in Fig. 3. After the accumulation of MB–dsDNA or MB–ssDNA complex on the HMDE surface, the electrode was transferred into the blank buffer solution for the measurement. The current signals of intercalated MB increased rapidly up until 100 nM and then tended to decrease at the dsDNA–MB-modified HMDE at higher concentrations (Fig. 3b). The difference between the signals obtained from the dsDNA–MB and the ssDNA–MB complexes (Fig. 3a) was obvious in all of the concentration levels of MB examined.

According to the reference method proposed by Millan and Mikkelsen [28], calibration data obtained at the ssDNA (Fig. 4A), DNA probe- (Fig. 4B) and DNA–DNA hybrid-modified (Fig. 4C), and bare GCEs (not shown) were used to estimate the partition coefficient of MB in the micro-environment near the GCE surface as in the equation below:

$$MB_{bound}/MB_{free} = (i_{bound} - i_{free})/i_{free}$$

where MB_{bound} and MB_{free} are the concentrations of bound and free MB complexes, respectively, i_{bound} is the voltammetric peak current obtained at the ssDNA- and probe-modified GCE, and i_{free} is the current obtained from the bare GCE. The validity of this equation depended on the following assumptions that were reported by Millan and Mikkelsen [28]: (a) the equilibration of the free and bound forms of MB occurred rapidly on the voltammetric time scale, (b) the diffusion coefficient of MB was the same in ssDNA and probe as in the bulk solution, (c) the preconcentration of MB bound to ssDNA at GCE surface did not significantly change the optimum MB complex concentration, and (d) the bound complex was electroinactive. The estimation of the partition coefficient of MB at probe- and ssDNA-modified GCEs was found as 0.76 and 0.92 by using the

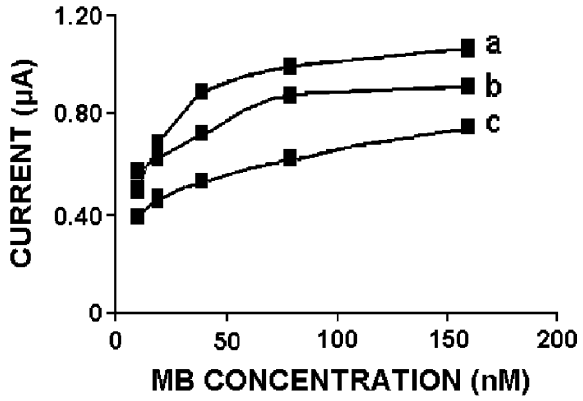

Fig. 2. Calibration plots of peak current against MB concentration, obtained with AdTSV at (a) ssDNA-modified HMDE, (b) bare HMDE, and (c) dsDNA-modified HMDE in 0.3 M ammonium formate, 50 mM phosphate buffer solution (pH 6.91). DNA immobilization: the dsDNA- or ssDNA-modified HMDE was prepared by immersing the bare HMDE into a 20-μl drop containing 10 ppm dsDNA or ssDNA, 0.3 M ammonium formate, 50 mM phosphate buffer solution (pH 6.91) for 2 min. MB accumulation: The electrode was subsequently washed with the buffer solution and transferred into the buffer solution, which contained MB. MB was accumulated onto the dsDNA- or ssDNA-modified HMDE at 200 rpm for 5 min. Measurement: The electrode was then subsequently washed with the buffer solution and transferred into the blank buffer solution for the voltammetric measurement. The cyclic voltammograms were collected at 500 mV/s scan rate. All CV measurements involved a fresh mercury surface. For the measurements performed with the bare HMDE, no DNA was adsorbed onto the electrode surface.

voltammetric peak currents obtained under the same conditions at ssDNA- and probe-modified and bare GCEs.

According to Fig. 4A and B, optimum ssDNA and DNA probe concentration for voltammetric measurements was found as 10 ppm. The MB signal almost remained constant after 10 ppm indicating that the full surface coverage of probe or ssDNA was achieved. The optimum DNA target concentration was found as 15 ppm in Fig. 4C, because the lowest MB signal was observed when the probe-modified CPE was exposed to 15 ppm target-containing solution. Thus, it was concluded that full surface coverage of the electrode surface with the hybrid was established with the target. There was a slight increase in the MB signal till 30 ppm indicating the fouling of the surface with the excessive single-stranded target oligonucleotides flanking at the surface. The detection limits, estimated from S/N = 3, correspond to 1.85 ng/ml ssDNA, 2.35 ng/ml DNA probe, and 2.03 ng/ml hybrid.

The effective discrimination against point mutation by using MB is displayed in Fig. 5. The response of the hybridization of the DNA probe (Fig. 5A-a) with the one-base mismatch-containing DNA oligonucleotide at CPE was detected by using MB. In the presence of a DNA oligonucleotide containing a one-base mismatch, the difference between the signal of the DNA probe-target hybridization (Fig. 5A-c) and the one of DNA probe-mismatch hybridization could slightly be observed (Fig. 5A-b). The hybrid formed with the one-base mismatch-containing oligonucleotide resulted in unbound two guanine bases. These accessible guanines increased the voltammetric signal of MB, when compared with the hybrid-modified CPE (Fig. 5A-c). Since the mismatched base was guanine, the increase in the MB signal was based on the close interaction of MB with the unbound guanine residues. The selectivity of the DNA probe for a mismatched DNA target sequence is shown to be poorer than that of the PNA probe, thus the MB signal resembled the signal obtained from the full DNA hybrid-modified CPE. Thus, PNA probes were employed for an effective detection of point mutation by using MB.

The response of the hybridization of the PNA probe (Fig. 5B-a) with the one-base mismatch-containing DNA oligonucleotide at CPE was detected by using MB. In the presence of a DNA oligonucleotide containing a one-base mismatch, which was nearly in the middle of this sequence, the difference between the signal of the PNA probe-target hybridization (Fig. 5B-c) and the one of PNA probe-mismatch hybridization could be observed (Fig. 5B-b). The hybrid formed with the one-base mismatch-containing oligonucleotide resulted in unbound two guanine bases. These accessible guanines greatly increased the voltammetric signal of MB, when compared with the hybrid-modified CPE (Fig. 5B-c). PNA probe did not form a hybrid with the mismatched target DNA oligonucleotide. The selectivity of the PNA probe for a complementary DNA sequence prevented the hybrid formation on the CPE surface, thus the MB signal after hybridization with the mismatch resembled the signal obtained from the PNA probe-modified CPE.

A series of three repetitive measurements of the reduction of MB resulted in reproducible results with an RSD of 9.85% for PNA probe-modified CPE, with an RSD of 9.20% for PNA–DNA mismatch-modified CPE, and an RSD of 10.45% for PNA–DNA hybrid-modified CPE. The detection limits, estimated from S/N = 3, correspond

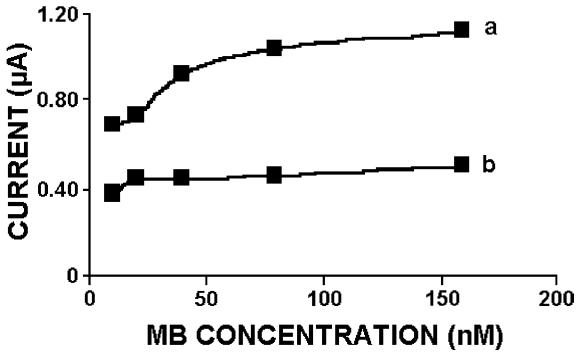

Fig. 3. Calibration plots of peak current against MB concentration obtained with AdTSV at HMDE with various concentrations of MB in the presence of (a) 10 ppm dsDNA and (b) 10 ppm ssDNA in 0.3 M ammonium formate, 50 mM phosphate buffer solution (pH 6.91). MB and DNA accumulation: HMDE was dipped into the buffer solution, which contained various concentrations of MB and DNA. Measurement: The electrode was then subsequently washed with the buffer solution and transferred into the blank buffer solution for the voltammetric measurement. The cyclic voltammograms were collected at 500 mV/s scan rate.

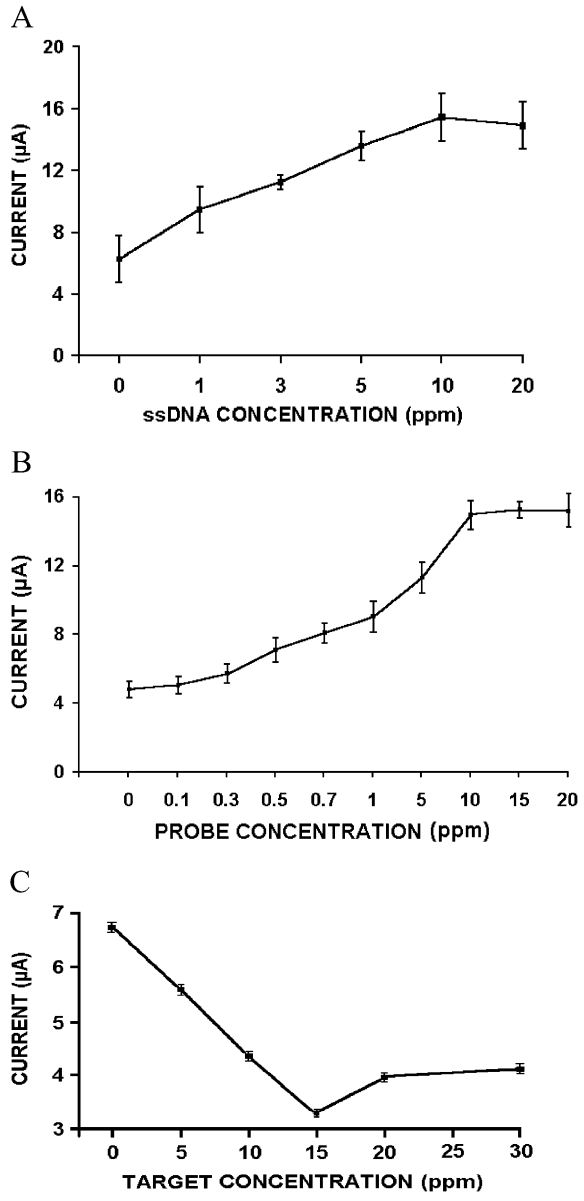

Fig. 4. Calibration plots of MB peak current against (A) ssDNA, (B) DNA probe, and (C) DNA target concentration. Ten parts per million ssDNA or DNA probe immobilization at pretreated CPE, 5 min at 0.50 V in 0.50 M acetate buffer (pH 4.80). Hybridization: 5 min at 0.50 V in 15 ppm one-base mismatch sequence or target containing 20 mM Tris–HCl buffer solution (pH 7.00). MB accumulation: 5 min, with 20 μM MB at 200 rpm without applying any potential in 20 mM Tris–HCl buffer (pH 7.00). Measurement by CV at 50 mV/s scan rate in 20 mM Tris–HCl buffer (pH 7.00).

to 1.82 ng/ml PNA probe and 2.75 ng/ml PNA–DNA hybrid at CPE. The differences in the current signals collected during the procedure above are shown in the inset for each signal as a column with error bars. A series of three repetitive measurements of the reduction of MB resulted in reproducible results with an RSD of 10.85% for DNA probe-modified CPE, with an RSD of 10.34% for PNA–DNA mismatch-modified CPE, and an RSD of 9.86% for PNA–DNA hybrid-modified CPE. The detection limits, estimated from S/N = 3, correspond to 2.56 ng/ml DNA probe and 2.91 ng/ml DNA–DNA hybrid at CPE. The differences in the current signals collected during the procedure above are shown in the inset for each signal as a column with error bars.

Control experiments were performed to assess whether the biosensor responds selectively, via hybridization, to the target. For example, Fig. 6 shows the response to the exposures of the PNA probe-modified CPE to 17-mer non-complementary DNA oligonucleotide. Following the exposure of the PNA probe-modified CPE (Fig. 6a) to the noncomplementary DNA oligonucleotide, a slight decrease in the reduction signal of MB was observed (Fig. 6b). PNA probes did not form any hybrid with the noncomplementary DNA oligonucleotide, because only a complementary

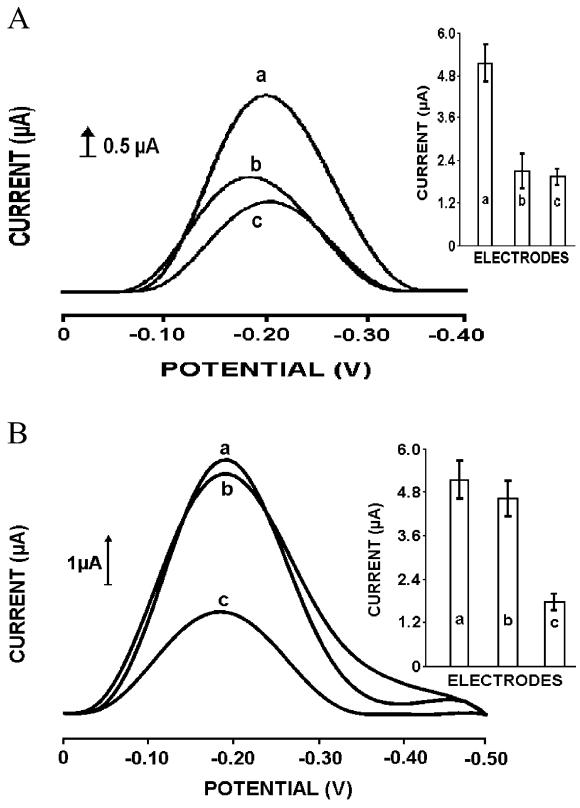

Fig. 5. (A) Differential pulse voltammograms for the reduction signals of MB in 0.50 M acetate buffer (pH 4.80) at (a) DNA probe-modified CPE, (b) after hybridization with one-base mismatch-containing DNA sequence, and (c) after hybridization with target DNA sequence. (B) Differential pulse voltammograms for the reduction signals of MB in 0.50 M acetate buffer (pH 4.80) at (a) PNA probe-modified CPE, (b) after hybridization with one-base mismatch-containing DNA sequence, (c) after hybridization with target DNA sequence. Ten parts per million DNA or PNA probe immobilization at pretreated CPE, 5 min at 0.50 V in 0.50 M acetate buffer (pH 4.80). Hybridization: 5 min at 0.50 V in 15 ppm one-base mismatch sequence or target containing 20 mM Tris–HCl buffer solution (pH 7.00). MB accumulation: 5 min, with 20 μM MB at 200 rpm without applying any potential in 20 mM Tris–HCl buffer (pH 7.00). Measurement by DPV with an amplitude of 10 mV at 20 mV/s scan rate in 20 mM Tris–HCl buffer (pH 7.00). Inset: the differences in the current signals collected in three repetitive DPV experiments following the procedure above for each column with error bars.

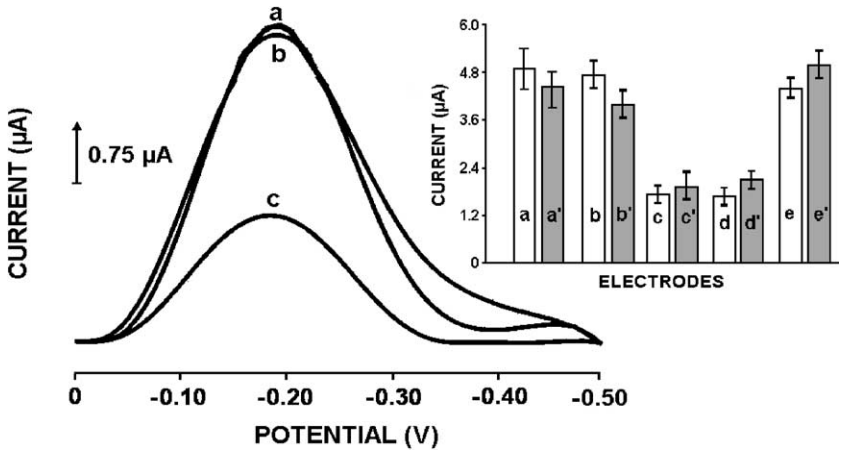

Fig. 6. Differential pulse voltammograms for the reduction signals of MB: (a) in PNA probe-modified CPE, (b) after hybridization with noncomplementary DNA sequence, (c) after hybridization with target DNA sequence. Inset: The differences in the current signals collected in three repetitive DPV experiments following the procedure above for each column with error bars. (a) PNA probe-modified CPE, (a′) DNA probe-modified CPE, (b) PNA probe after hybridization with noncomplementary DNA sequence, (b′) DNA probe after hybridization with noncomplementary DNA sequence, (c) PNA probe after hybridization with target DNA sequence, (c′) DNA probe after hybridization with target DNA sequence, (d) PNA probe after hybridization with a mixture of 15 ppm target and 15 ppm mismatch-containing DNA sequence, (d′) DNA probe after hybridization with a mixture of 15 ppm target and 15 ppm mismatch-containing DNA sequence, (e) PNA probe after hybridization with 292 ppm noncomplementary DNA sequence, (e′) DNA probe after hybridization with 292 ppm noncomplementary DNA sequence. Other conditions as in Fig. 5.

sequence could have formed a hybrid at the CPE surface as in Fig. 6c. More decrease in the MB signal of DNA probe (Fig. 6, inset b′) was observed upon hybridization with the DNA noncomplementary sequence than that was observed for PNA probe. The signal obtained from DNA–DNA hybrid (Fig. 6, inset c′) was also higher than that observed for PNA–DNA hybrid (Fig. 6, inset c). The difference between the probe and hybrid signals could be better observed with PNA probes. When the PNA probe was challenged with a mixture, which contained equal amount of mismatch and target, the hybrid signal was again just as high as the hybrid signal obtained with only the target (Fig. 6, inset d). When the DNA probe was exposed to the mixture of mismatch and target (Fig. 6, inset d′), the hybrid signal was affected by the presence of mismatch and was higher than that observed with PNA probe. The PNA probe signal did not change much when challenged with an excessive amount of noncomplementary DNA sequence (Fig. 6, inset e). The DNA probe signal was found to be increasing when exposed to an excessive amount of noncomplementary DNA sequence indicating that nonspecific binding occurred at the surface (Fig. 6, inset e′). Thus, PNA probes were found to be more specific to the target sequences than the DNA probes.

A series of three repetitive measurements of hybridization of the 10 ppm PNA probe with the 15 ppm 17-mer noncomplementary oligonucleotide resulted in reproducible results and nearly no decrease in the response with an RSD of 8.35%, the hybridization of the 10 ppm DNA probe with the 15 ppm 17-mer noncomplementary DNA oligonucleotide resulted in reproducible results and nearly no decrease in the response with an RSD of 9.54%, the hybridization of the 10 ppm PNA probe with the 15 ppm target resulted in reproducible results and nearly no decrease in the response with an RSD of 7.56%, the hybridization of the 10 ppm DNA probe with the 15 ppm target resulted in reproducible results and nearly no decrease in the response with an RSD of 7.85%, the hybridization of the 10 ppm PNA probe with the 15 ppm target and the 15 ppm mismatch-containing DNA sequence resulted in reproducible results and nearly no decrease in the response with an RSD of 9.15%, the hybridization of the 10 ppm PNA probe with the 15 ppm target and the 15 ppm mismatch-containing DNA sequence resulted in reproducible results and nearly no decrease in the response with an RSD of 9.92%, the hybridization of the 10 ppm PNA probe with the 292 ppm 17-mer noncomplementary oligonucleotide resulted in reproducible results and nearly no decrease in the response with an RSD of 10.54%, the hybridization of the 10 ppm PNA probe with the 292 ppm 17-mer noncomplementary oligonucleotide resulted in reproducible results and nearly no decrease in the response with an RSD of 8.79%.

4. Conclusion

This report demonstrates that MB, in connection with PNA probes, is suitable for detecting DNA hybridization and point mutation in connection with different voltammetric techniques. The increase in the signal of MB at the ssDNA-modified electrodes indicated the interaction of MB guanine bases. Electrochemical DNA biosensors are characterized by its simplicity, reliability, and small sample requirements. DNA-modified electrodes offer a valuable tool to examine the redox behavior of species within the DNA environment. MB proves to be a promising electrochemical hybridization

indicator. Future work in this laboratory will focus on employing MB as electrochemical hybridization indicator for the detection of clinically important oligonucleotides by using DNA biosensor.

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