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DNA Biosensor for *Microcystis* spp. Sequence Detection by Using Methylene Blue and Ruthenium Complex as Electrochemical Hybridization Labels

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An electrochemical biosensor for the voltammetric detection of DNA sequences related to the bloom-forming genera of cyanobacteria, *Microcystis* spp., is described. A specific DNA "probe" is designed based on sequence polymorphism within the 16S ribosomal DNA (rDNA) of the *Microcystis* spp. These single-stranded probes were immobilized onto carbon paste electrode (CPE) with the adsorption at a controlled potential. The probes were hybridized with the complementary "target" sequences at the electrode. The formed hybrids on the electrode surface were evaluated by differential pulse voltammetry (DPV) and cyclic voltammetry (CV) using methylene blue (MB) and tris (2,2' – bipyridine) ruthenium (II) chloride ($[Ru(bpy)_3]^{2+}$) as the labels of hybridization. The response of the probe modified CPE to the exposure of the non–complementary oligonucleotide proves the specificity of the hybridization with the target. The two-bases mismatch could also be discriminated and specific detection of *Microcystis* spp. was achieved by using the difference between the voltammetric peaks of MB and $[Ru(bpy)_3]^{2+}$obtained with the probe and hybrid-modified CPEs. The detection of *Microcystis* spp. target DNA from real tap water and river water samples was also achieved.

Key Words: DNA Biosensor, *Microcystis* spp., Methylene Blue, Ruthenium Bipyridine.

Introduction

DNA biosensor technologies are currently under intense investigation owing to their great promise for the rapid and low-cost detection of specific DNA sequences in human, viral and bacterial nucleic acids. As the sequencing of the human genome continues, the mutations responsible for numerous inherited human disorders are now mapped[1−4]. Pathogens responsible for disease states, bacteria and viruses are also detectable via their unique nucleic acid sequences and interest in their detection continues to grow[5−7].

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The development of DNA hybridization biosensors holds great promise for obtaining sequence-specific information in connection with clinical, environmental or forensic investigations. Electrochemical transducers offer a very attractive route for converting the hybridization event into a useful analytical signal[8−15]. Such electrochemical DNA biosensors rely on the immobilization of a single-stranded (ss) DNA sequence (the "probe") for hybridizing with its complementary "target" strand to give rise to voltammetric or potentiometric signals (in connection to with a suitable electrochemical label)[15−22].

Mycrocystins are a family of cyclic polypeptides produced by different species of cyanobacteria (blue-green algae), which can form blooms in lakes and reservoirs[23]. Their basic structure is a cyclic heptapeptide and their variations give rise to more than 50 types of microcystins known today[23].

Microcystins and related polypeptides are potent hepatotoxins in fish, birds and mammals[24]. The consequence of acute poisoning by these compounds is a rapid desorganization of the hepatic architecture[24,25] leading to massive intrahepatic hemorrhage, often followed by death of the animal by hypovolemic shock or hepatic insufficiency[26]. Matsushima *et al.*[27] observed that microcystins penetrate with difficulty into the epithelial cells, which reflects tissue specificity, and their target cell is the hepatocyte. This cellular specificity and organotropism of microcystins is due to the selective transport system, the multispecific bile acid transport system, present only in hepatocytes[25,28].

Microcystins are potent inhibitors of protein phosphatases 1 (PP1) and 2A (PP2A)[29−31], which are regulatory enzymes present in the cytosol of mammalian cells. This action may explain the effects of microcystins as cancer promoters[32,33] and the promotion of primary liver cancer in humans exposed to long-term low doses of these cyclic peptide toxins through drinking water[34−36] as well as the cytoskeletal disruption and formation of plasma membrane blebs (blebbing) in hepatocytes[35].

Since microcystins are potent hepatotoxins for humans and animals, the development of sensitive and reliable detection methods is of great importance. Efforts have been made to develop more sensitive screening methods to replace the nonspecific mouse bioassay, traditionally used for the identification of toxic strains of *Microcystis*. The bloom-forming genera of cyanobacteria, *Microcystis* spp., produce toxins including hepatotoxic microcystins that are potential health hazards for livestock and humans[37−38].

DNA biosensors can detect the presence of genes or mutant genes associated with inherited human diseases[2]. They can be employed for determining early and precise diagnoses of infectious agents in various environments[3]. These devices can be exploited for monitoring sequence-specific hybridization events directly[8] or by DNA intercalators (metal coordination complexes, etc.) that form complexes with the nitrogenous bases of DNA[9−12]. Most label-based electrochemical DNA biosensors use cationic metal complexes (e.g., $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$)[39−42] that interact in a different way with ss-DNA and ds-DNA. Some of the ruthenium complexes of polypyridyl ligands exist as chiral molecules capable of enantioselective recognition of guanine bases in DNA. Ruthenium (II) complexes with 1,10-phenanthroline $[Ru(phen)_3]^{2+}$ and related ligands have been studied extensively as structural probes and mediators of DNA cleavage reactions[42−46].

Methylene blue (MB) is an aromatic heterocycle that binds strongly to DNA via intercalation. MB interacts in a different way with ssDNA and dsDNA. The decreased electrochemical response due to the association of the large planar hydrophobic phenothiazine group with the surface duplex thus serves as the DNA recognition signal[47,48]. In particular, the cationic charge in MB would improve the DNA binding affinity electrostatic interaction with a phosphate backbone. Kelley and Barton[49] reported on a gold electrode derivatized with 15-base pair double-stranded DNA oligonucleotides containing a pendant 5'-hexanethiol

linker. The use of MB as the hybridization label for the detection of short DNA sequences related to the hepatitis B virus is reported[50,51]. Evidence of the direct interaction of MB with guanine based on DNA coated carbon paste electrodes (CPEs) was also investigated by Yang *et al.*[52]. Rohs *et al.*[53] reported a modeling study for MB binding to DNA with an alternating guanine–cytosine base sequence. Enescu *et al.*[54] investigated the conformation of MB–guanine complexes 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. Tani *et al.*[55] reported a shift in the peak potentials of the square wave voltammetric signals of MB obtained from the thiol terminated oligonucleotides self assembled on a gold electrode. The peak potential of the MB signal at the thiol terminated probe–modified gold electrode was 10-15 mV more positive than the one at the thiol terminated hybrid modified gold electrode. Recently, Yan *et al.*[56] have reported the use of MB and $[Ru(bpy)_3]^{2+}$ for the determination of a specific gene related to *Microcystis* spp. adsorbed on a gold electrode. A voltammetric hybridization detection scheme on the self–assembled monolayer of alkanethiol modified gold electrodes by using MB as the electrochemical label was described in our laboratory[57].

Here we describe a voltammetric electrochemical biosensor for the specific detection of short DNA sequences related to *Microcystis* spp. by using external redox labels, MB and $[Ru(bpy)_3]^{2+}$. The new *Microcystis* spp. electrochemical biosensor relies on the immobilization of a 17-base single stranded (ss) oligonucleotide probe designed based on sequence polymorphism within the 16S ribosomal DNA (rDNA) of *Microcystis* spp.[58]. The formed hybrids on the electrode surface were evaluated by differential pulse voltammetry (DPV) and cyclic voltammetry (CV) using MB and $[Ru(bpy)_3]^{2+}$ as the labels of hybridization reaction.

The features of the method are discussed and compared with those of methods previously reported in the literature.

Experimental Section

Apparatus

Differential pulse voltammetry (DPV) was performed with a Autolab PGSTAT 30 electrochemical analysis system, with the GPES 4.8 software package (Eco Chemie B. V., Utrecht, The Netherlands). A PowerLab voltammetric unit (ADInstruments, Australia) in connection with an EG& G PAR 264A potentiostat (Princeton, USA) was used to obtain the cyclic voltammograms. For each voltammetric measurement, a three electrode system, consisting of a carbon paste electrode (CPE), reference electrode (Ag/AgCl) and platinum wire as the auxiliary electrode, joined the cell. The body of the working electrode was a glass tube (3 mm i.d.) tightly packed with carbon paste. Electrical contact was provided by a copper wire. 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 of the working electrode was polished on a weighing paper to a smooth finish before use.

Chemicals

The 17-base synthetic oligonucleotides were purchased from Synthegen, LLC (Houston, Texas, USA); their base sequences are as below:

target (17-base sequence A):

5′-TAA-GCA-ACC-TGA-TTT-GA- **3′**

immobilized probe (17-base sequence B):

5′-TCA-AAT-CAG-GTT-GCT-TA- **3′**

Two-bases mismatch (17- base sequence A′):

5′- TAA-GCA-A**GG**-TGA-TTT-GA- **3′**

noncomplementary (21-base sequence C):

5′- AAC-GTG-TGA-ATG-ACC-CAG-TAC- **3′**

The 17-base sequence A is complementary to 17-base sequence B; 17-base sequence A′ is a mutant of the 17-base sequence A with two bases changed, as indicated by the underlines. All oligonucleotide stock solutions of the 17-base oligomers (100 mg/L) were prepared with TE solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.00) and kept frozen. More dilute solutions were prepared with 0.5 M acetate buffer (pH 4.80). Methylene blue was purchased from the La Pine Scientific Company (USA). $[Ru(bpy)_3]^{2+}$ was purchased from Sigma (Germany). Other chemicals were of analytical reagent grade. Sterilized, deionized water was used in all solutions. A spectrophotometer (UV–160A, Schimadzu, Japan) was employed to correct the concentration of nucleic acids by measuring optical density ($\lambda = 260$ nm). All glassware, containers, pipette tips and the cell (with exception of the electrodes) were sterilized by autoclaving for 30 min. In-house distilled water was used to rinse the electrodes prior to use.

Procedure

Each measurement involved the immobilization/detection cycle at a fresh carbon paste surface. All the experiments were performed at room temperature (25.0 ± 0.5°C).

The *Microcystis* spp. sequence detection basically consisted of four steps: probe immobilization, hybridization, label binding, and voltammetric transduction. During the electrode transfer to the next solution, the surface was rinsed with distilled water.

Probe immobilization: The CPE was activated by applying +1.70 V for 1 min in 0.05 M phosphate buffer solution (pH 7.40) (PBS) without stirring. The probe was subsequently immobilized on a pretreated CPE by applying a potential of +0.50 V for 5 min in the stirred 10 μg/mL probe containing 0.50 M acetate buffer solution (pH 4.80) with 20 mM NaCl (ABS). The electrode was then rinsed with blank ABS for 10 s.

Hybridization: Hybridization was performed by dipping the electrode into the stirred 15 μg/mL target containing 20 mM Tris-HCl buffer (pH 7.00) solution with 20 mM NaCl (TBS) for 5 min while holding the potential at +0.50 V. The electrode was then rinsed with blank ABS for 10 s. The same procedure as above was repeated for the hybridization with mismatch and also noncomplementary sequences.

Label binding to the hybrid: MB or $[Ru(bpy)_3]^{2+}$ was accumulated onto the surface hybrid by immersing the electrode into the stirred TBS containing 20 μM MB or $[Ru(bpy)_3]^{2+}$ as a hybridization label for DPV measurements and 1 mM MB or $[Ru(bpy)_3]^{2+}$for CV measurements for 5 min while holding the potential at +0.50 V. After accumulation of MB or $[Ru(bpy)_3]^{2+}$, the electrode was rinsed with blank TBS for 10 s.

Voltammetric transduction: The accumulated MB or $[Ru(bpy)_3]^{2+}$ was measured by using DPV and CV in the blank TBS. The raw data were also treated using the Savitzky and Golay filter (level 2) of

the GPES software, followed by 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.

Voltammetric detection of *Microcystis* spp. from real samples: Real samples were collected from tap water in our laboratory and river water from Kaynaklar River. The real samples were diluted with the TBS in a 1:1 ratio. The 15 μg/mL target DNA was spiked into these real samples. The probe modified CPE was dipped into the target DNA containing the real sample. The other conditions were as described above.

Results and Discussion

The new biosensor relies on the electrochemical transduction of the hybridization between the short complementary *Microcystis* spp. strand sections. The detection of hybridization is accomplished using label species, such as MB and $[Ru(bpy)_3]^{2+}$, which exhibits, a reversible redox couple and interact with the guanine residues on the immobilized probe and hybrid at the electrode surface[41−52].

The overall performance of the biosensor is strongly dependent upon experimental variables influencing the hybridization efficiency, such as temperature, ionic strength, or probe length. The influence of experimental parameters including salt concentration and accumulation time was also explored for optimum analytical performance[15]. It has been reported that the higher association constant obtained at 20 mM NaCl is expected, since the binding reaction is electrostatic in nature, and electrostatic association is favored at low ionic strength[20].

The concentration of the labels has a pronounced effect on the hybridization response (not shown). The response increases sharply with the MB and $[Ru(bpy)_3]^{2+}$ concentration up to 20 μM, above which it starts to level off; 20 μM of the label solutions was chosen to be the optimum concentration for further hybridization detection schemes.

Figure 1 shows differential pulse voltammograms for the MB (Figure 1A) and $[Ru(bpy)_3]^{2+}$ (Figure 1B) label at the 17-base *Microcystis* spp. only probe-modified CPE (a), after hybridization with target (complementary) oligonucleotide (sequence A) (c) and with no oligonucleotides on the CPE (b). A significant increase in the voltammetric peaks is observed for the labels, showing that the MB and $[Ru(bpy)_3]^{2+}$ interact with the guanine residues on the immobilized probe. The free guanine bases increased MB and $[Ru(bpy)_3]^{2+}$ signals, because these labels could accumulate at the probe modified electrode surface.

A series of five repetitive measurements of the reduction of MB resulted in reproducible results with a relative standard deviation (RSD) of 10.80% for probe modified CPE, an RSD of 9.20% for hybrid modified CPE and an RSD of 9.73% for bare CPE. The detection limits, estimated from S/N = 3, correspond to 2.84 ng/mL probe and 5.64 ng/mL hybrid, respectively. A series of five repetitive measurements of the oxidation of $[Ru(bpy)_3]^{2+}$ resulted in reproducible results with an RSD of 9.78% for probe modified CPE, an RSD of 10.12% for hybrid modified CPE and an RSD of 8.37% for bare CPE. The detection limits, estimated from S/N = 3, correspond to 1.34 ng/mL probe and 2.94 ng/mL hybrid, respectively.

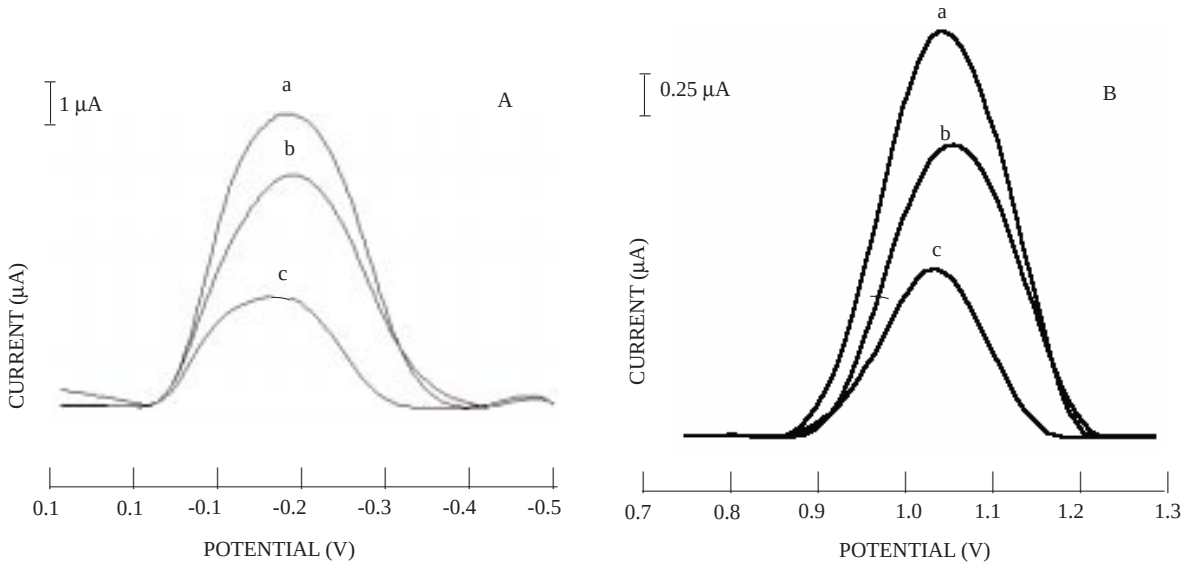

Figure 1. Differential pulse voltammograms for the **[A]** 20 μM MB and **[B]** 20 μM $[Ru(bpy)_3]^{2+}$ label at the 17-base *Microcystis* spp. probe (sequence B)-immobilized electrode; **(a)** before hybridization, **(b)** at bare CPE, **(c)** after hybridization with 17-base *Microcystis* spp. target (sequence A). CPE pretreatment, 1 min at +1.70 V in PBS; probe immobilization, 5 min at +0.5 V in stirred 10 μg/mL probe (sequence B) containing ABS; hybridization, 5 min at +0.50 V in stirred 15 μg/mL target (sequence A) containing TBS; label binding, 5 min at +0.50 V in TBS containing 20 μM MB or $[Ru(bpy)_3]^{2+}$; measurements of accumulated MB or $[Ru(bpy)_3]^{2+}$in blank TBS by using DPV with 10 mV pulse amplitude.

Figure 2 shows that the substantial response of the two-bases mismatch oligonucleotide at the DNA biosensor is also detected by using MB (Figure 2Aa) and $[Ru(bpy)_3]^{2+}$ (Figure 2Ba). In the presence of an oligonucleotide containing a two-base mismatch (sequence A*ı*) that included guanine bases nearly in the middle of this sequence, the difference between the signal of the sequence A-sequence B hybridization and the one of the sequence A*ı*-sequence B hybridization were observed. The signal obtained at the mismatch-modified CPE was greater than that at the probe-modified CPE, because the mismatched bases were guanine and the close interaction of these labels with guanine bases increased the voltammetric signal. The labels could accumulate more at the probe modified surface than at the hybrid modified electrode surface. The labels reported here are capable of selectively discriminating against mismatches, as desired for the detection of disease-related point mutation in the guanine bases of the cyanobacteria. The resulting biosensors offer great promise for mismatch-sensitive hybridization detection and can operate over a wide range of hybridization conditions.

Figure 3 shows cyclic voltammograms for the MB (Figure 3A) and $[Ru(bpy)_3]^{2+}$ (Figure 3B) label at the 17-base *Microcystis* spp. probe-modified CPE (a) after hybridization with (b) target (complementary) oligonucleotide (sequence A) and (c) two-bases mismatch oligonucleotide (sequence C). MB and $[Ru(bpy)_3]^{2+}$ show much higher signals at probe modified electrodes because of the exposed and unbound guanine bases. Since the guanine bases were covered with the complementary cytosine bases, the MB and $[Ru(bpy)_3]^{2+}$signals greatly decreased.

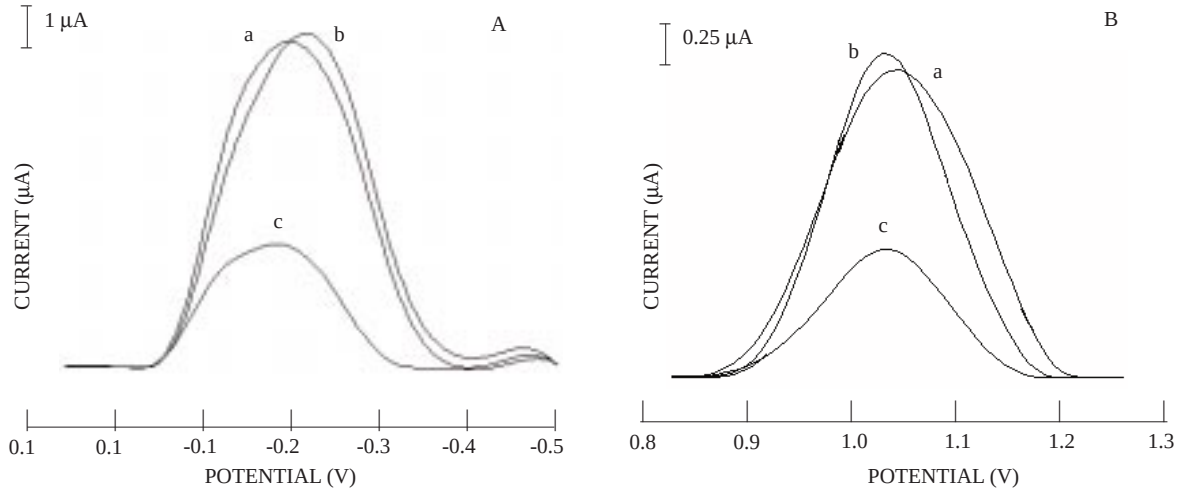

Figure 2. Differential pulse voltammograms for **[A]** 20 μM MB and **[B]** 20 μM $[Ru(bpy)_3]^{2+}$label at the 17-base *Microcystis* spp. probe (sequence B)-immobilized electrode; **(a)** before hybridization; **(b)** after hybridization with the two-bases mismatch oligonucleotide (sequence A′), (c) after hybridization with 17-base *Microcystis* spp. target (sequence A). Other conditions are as in Figure 1.

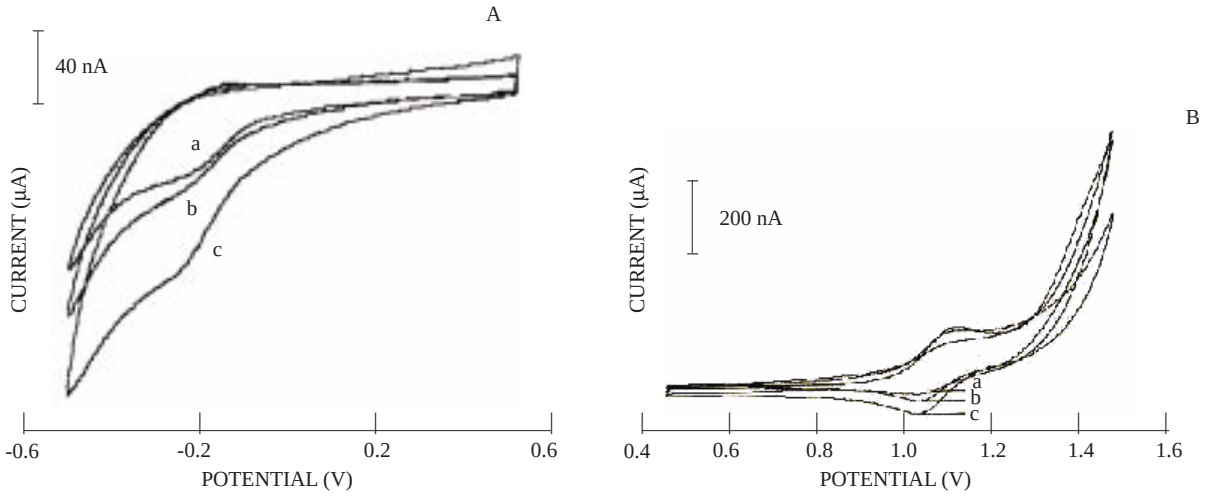

Figure 3. Cyclic voltammograms for the **[A]** 1 mM MB and **[B]** 1 mM $[Ru(bpy)_3]^{2+}$ label at the 17-base *Microcystis* spp. probe (sequence B)-immobilized electrode, **(a)** before hybridization; **(b)** after hybridization with the two-bases mismatch oligonucleotide (sequence A′), (c) after hybridization with 17-base *Microcystis* spp. target (sequence A). CPE pretreatment, 1 min at +1.70 V in PBS; probe immobilization, 5 min at +0.50 V in stirred 10 μg/mL probe (sequence B) containing ABS; hybridization, 5 min at +0.50 V in stirred 15 μg/mL target (sequence A) containing TBS; label binding, 5 min at +0.50 V in TBS containing 1 mM MB or $[Ru(bpy)_3]^{2+}$; measurements of accumulated MB or $[Ru(bpy)_3]^{2+}$in blank TBS by using CV.

Control experiments were performed to assess whether the *Microcystis* spp. sensor responds selectively, via hybridization, to the target. For example, Figure 4 shows the response to sequential exposures of the CPE to the target (a) and to a 17-base noncomplementary oligonucleotide (b). Voltammetric peaks of

hybridization labels MB (Figure 4A) and $[Ru(bpy)_3]^{2+}$ (Figure 4B) that were approaching the probe were observed following exposure of the probe immobilized electrode to the noncomplementary oligonucleotides (sequence C) under the same hybridization conditions. These observations demonstrate that effective discrimination against noncomplementary oligonucleotides is achieved with the labels reported here and binding between the complementary sequences, which is primarily via hybridization, could be monitored. The slight decrease in the voltammetric response of the noncomplementary oligonucleotide is attributed to the possible binding of some of the complementary bases during the hybridization reaction between sequences B and C.

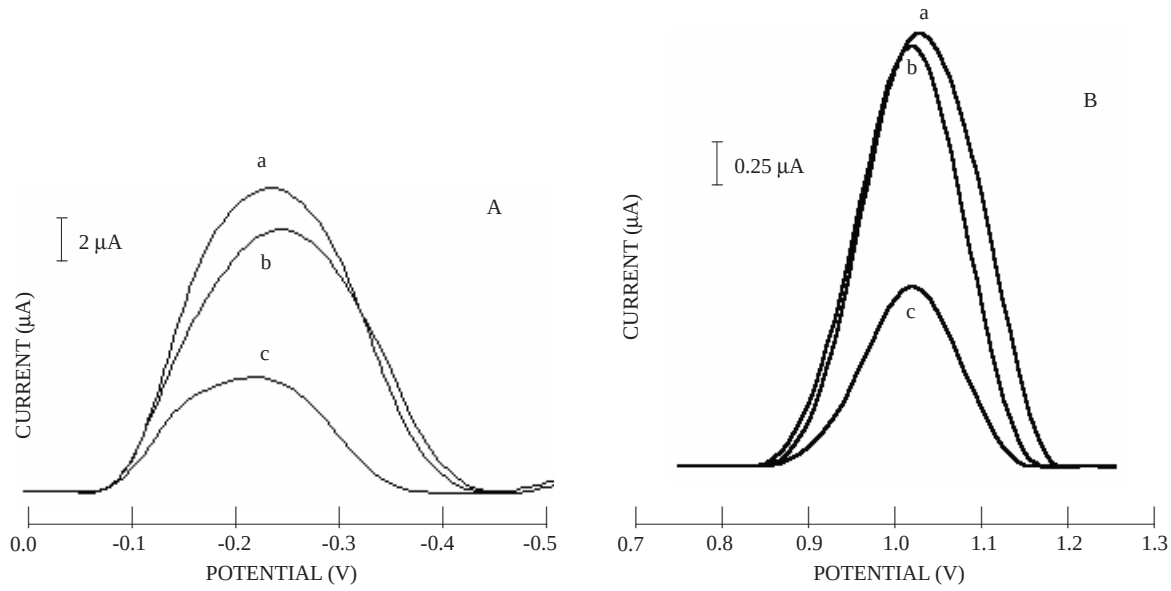

Figure 4. Differential pulse voltammograms for **[A]** 20 μM MB and **[B]** 20 μM $[Ru(bpy)_3]^{2+}$label at the 17-base *Microcystis* spp. probe (sequence B)-immobilized electrode; **(a)** before hybridization, **(b)** after hybridization with the noncomplementary oligonucleotide (sequence C), **(c)** after hybridization with 17-base *Microcystis* spp. target (sequence A). Measurements of accumulated label in blank TBS by using DPV. Other conditions are as in Figure 1.

The effect of target concentration on the MB (Figure 5A) and $[Ru(bpy)_3]^{2+}$ (Figure 5B) signals was observed. When the target concentration for the hybridization with 10 μg/mL probe was increased until 5 μg/mL target, the MB signal (Figure 5A) decreased linearly. The regression line for the target DNA by using MB was y = -0.0123x + 14.78 (R^2 = 0.9968). The regression line for the target DNA by using $[Ru(bpy)_3]^{2+}$ was y = -0.0141x + 11.43 (R^2 = 0.9945). When the probe was exposed to target concentrations 5 μg/mL or above the MB signal remained almost constant. In case of $[Ru(bpy)_3]^{2+}$ (Figure 5B), when the target concentration for the hybridization with 10 μg/mL probe was increased, the $[Ru(bpy)_3]^{2+}$ signal (Figure 5B) decreased until 10 μg/mL target. When the probe was exposed to target concentrations of 10 μg/mL or above, the $[Ru(bpy)_3]^{2+}$ signal remained almost constant. Thus, an optimum target concentration of 15 μg/mL was employed.

The effect of scan rate on the CV signals of MB[51] and $[Ru(bpy)_3]^{2+}$ (Figure 6A) at the ssDNA-modified CPE was also investigated. As shown in Figure 6B, the behavior of the label was based on diffusion until 50 mV/s because the current peaks increased linearly with the square root of the scan rate. After 50 mV/s,

adsorption began to take effect because the signals remained almost constant. The plot for the square root of scan rate against $[Ru(bpy)_3]^{2+}$ signals (Figure 6B) remained almost constant after 50 mV/s indicating adsorptional behavior. The regression line for MB was y = -0.0112x + 11.32 (R^2 = 0.9936). The regression line for $[Ru(bpy)_3]^{2+}$ was y = -0.0156x + 15.34 (R^2 = 0.9947).

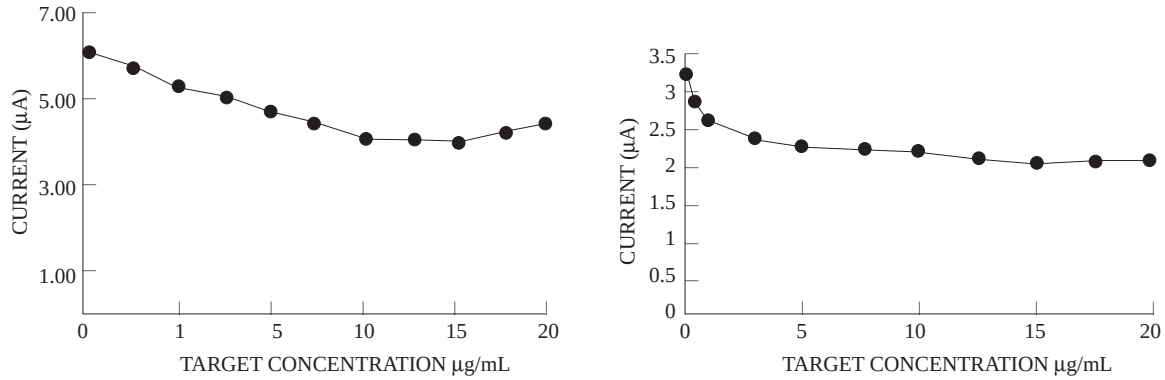

Figure 5. Calibration plots of **(A)** MB and **(B)** $[Ru(bpy)_3]^{2+}$peak current aganist target concentration. Other conditions are as in Figure 1.

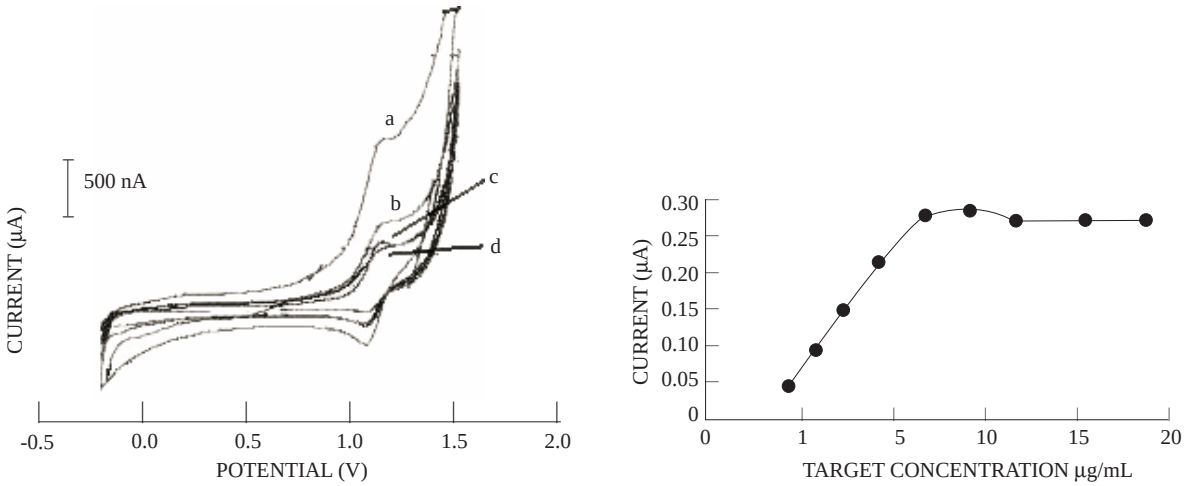

Figure 6. (A) Cyclic voltammograms for 1 mM $[Ru(bpy)_3]^{2+}$ label at ssDNA modified CPE with the scan rates **(a)** 200, **(b)** 100, **(c)** 50, **(d)** 20 mV/s; **(B)** Scan rate dependence of $[Ru(bpy)_3]^{2+}$ signals obtained with CV at ssDNA modified CPE with the square root of scan rates. CPE pretreatment, 1 min at +1.70 V in blank PBS; ssDNA immobilization, 5 min at +0.5 V in stirred 10 μg/mL ssDNA containing ABS; label binding, 5 min at +0.50 V in TBS containing 1 mM $[Ru(bpy)_3]^{2+}$; measurement of accumulated $[Ru(bpy)_3]^{2+}$ by CV at various scan rates in blank TBS.

Figure 7A displays the cyclic voltammograms of $[Ru(bpy)_3]^{2+}$ obtained from the probe-modified CPE after hybridization in real samples of tap water. The probe signal obtained from tap water containing TBS showed a high signal as expected (Figure 7A-a). The hybrid signal obtained from tap water samples (Figure 7A-b) was higher than that obtained from target containing TBS with no water sample (Figure 7A-c). The increase in the hybrid signal from real samples was attributed to the different chemical and biological

interferences that might be in the tap water. These interferences prevented hybridization from occurring efficiently. Figure 7B-a displays the cyclic voltammograms of $[Ru(bpy)_3]^{2+}$ obtained from the probe-modified CPE in river water containing TBS. The hybrid signal obtained from tap water samples (Figure 7A-b) was higher than that obtained from target containing TBS with no water sample (Figure 7A-c). The river water effectively prevented hybridization, because more interferences were present in the river water than in the tap water.

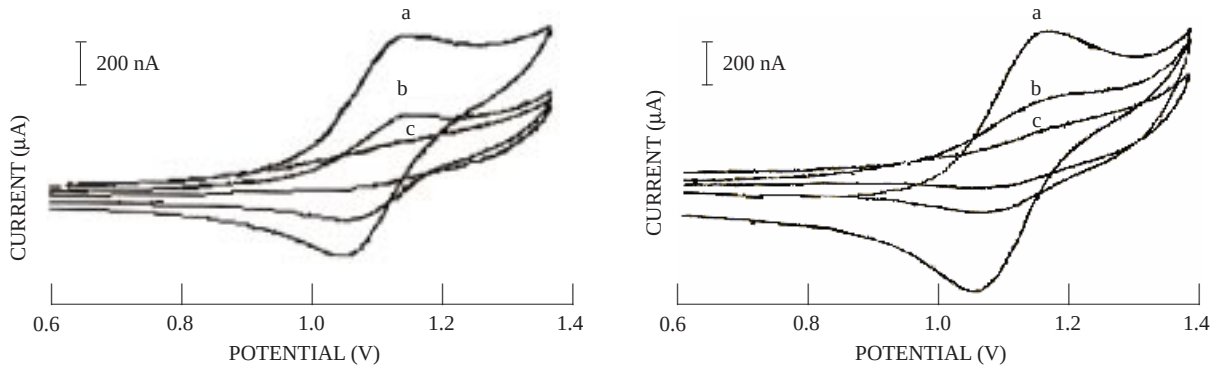

Figure 7. Cyclic voltammograms for 1 mM $[Ru(bpy)_3]^{2+}$ label in **(A)** tap water samples and in **(B)** river water samples; Other conditions as in Figure 3, except the hybridization was performed in both TBS where no tap or river water was spiked and TBS spiked with tap or river water in 1:1 ratio. **(a)** probe modified CPE after hybridization in real samples containing TBS with no target DNA, **(b)** hybrid in TBS with real samples with 15 μg/mL target DNA, **(c)** hybrid with 15 μg/mL target DNA in TBS.

Conclusion

The identification and detection of microorganisms in aquatic environments is important in environmental monitoring, clinical and food industrial fields. In this contribution, we have described an electrochemical hybridization biosensor for the detection of short fragments of *Microcystis* spp. DNA. Such a use of electrochemical DNA probes can decrease the time and cost of *Microcystis* spp. screening. While the concept has been demonstrated in connection with one oligonucleotide related to the *Microcystis* spp. DNA 17-mer sequence, further improvements may be achieved by using several probes from different regions, in connection with multielectrode array and multiple hybridization events. The procedure we report here is simple, economical and provides rapid detection. DNA biosensors eliminate the use of radioisotopes, and require significantly shorter hybridization times. Similar developments should address the growing needs for the decentralized testing of environmental or water samples for the *Microcystis* spp. pathogen.

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