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Brilliant cresyl blue as electroactive indicator in electrochemical DNA oligonucleotide sensors

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ABSTRACT

A new electrochemical DNA biosensor is presented based on carbon past electrode (CPE) for immobilization and detection of short DNA sequences with brilliant cresyl blue (BCB) as electroactive label. The interaction of BCB with DNA is electrochemically detected and BCB displays different signals in the interaction to ssDNA and dsDNA and variation in the BCB signal represents the extent of hybridization at the electrode surface. The effect of solution pH on electrochemical behavior of BCB was investigated. Additionally, the effect of solution pH on BCB accumulation on the CPE was studied. Furthermore, experiments showed that the solution pH could influence the differential pulse voltammetry (DPV) signal of BCB accumulated on the electrode and the highest BCB signal was obtained in pH 7.00. The effect of electrochemical pretreatment of CPE on the ability of electrode in probe adsorption, BCB accumulation and conditions of probe immobilization including potential and time was investigated and optimum conditions were suggested. The peak currents of BCB were linearly related to the concentration of the target oligonucleotide sequence in the range of 1.0×10^{-8} to 5.0×10^{-6} M. The detection limit of this approach was 9.00 nM. The selectivity of the biosensor was studied using noncomplementary oligonucleotide.

1. Introduction

The use of DNA recognition layers represents an exciting development in analytical chemistry. Among the various sensing devices developed so far, electrochemical biosensors have received a great deal of attention due to their high sensitivity and rapid speed of detection. In addition, electrochemical techniques are ideally suitable for miniaturization and have the potential to simplify nucleic acid analysis using low cost electronics [1].

The other important feature about DNA is its interaction with biological compound and drugs that can lead to advances in pharmacology and diagnosis basis of many diseases [2,3].

Electrochemical detection of DNA hybridization is classified into two direct and indirect methods. Signal transduction induced directly from oxidation of guanine or adenine moieties in DNA strands (label-free detection) makes the principle of DNA hybridization detection in direct strategy [4–7], while indirect DNA hybridization detection method is based on incorporation of an electroactive label [8–11]. Electroactive indicators include anticancer agents [12], organic dyes [13] and metal complexes [14].

Brilliant cresyl blue (BCB), one of the organic dyes, was commonly used in various biological studies such as usefulness of BCB staining as an auxiliary method of screening for α-thalassemia [15], supplementation with cysteamine during maturation and embryo culture on embryo development of prepubertal goat oocytes selected by the BCB test [16], developmental competence of heifer oocytes selected using the BCB test [17], developmental competence of prepubertal goat oocytes selected with BCB and matured with cysteamine supplementation [18], and selection of prepubertal goat oocytes using the BCB test [19]. BCB belongs to quinine-imide dyes with planar structure and it is positively charged in neutral solutions. BCB was also used to determine heparin [20], oxalate [21], nitrite [22], protein [23], oxalic acid [24] and hydrazine [25] through its photochemical property. BCB could bind with DNA by electrostatic attraction, which was used for determination of DNA in spectroscopic method such as molecular spectroscopy study of the reaction of nucleic acids with BCB [26]. BCB as a new red region fluorescent probe for determination of nucleic acids [27] and binding behavior of BCB to calf thymus DNA was studied by spectrophotometric and voltammetric methods [28].

Recently, the use of BCB for detection of DNA hybridization has been reported by resonance light scattering method [29]. However, to our knowledge, application of BCB as an electrochemical indicator has never been devoted to fabrication of DNA hybridization sensors by electrochemical method and carbon paste electrode.

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We have already reported detection of IL-2 corresponding oligonucleotides using a non-inosine substituted probe on pencil graphite electrode (PGE) by direct and indirect methods [10,30,31]. In the indirect method we used methylene blue as the electroactive label. The present study aims to elucidate the electrochemical behavior of BCB as an electroactive label for electrochemical DNA biosensors. To take full advantage of this label, it has also been attempted to employ BCB for the development of electrochemical based DNA biosensors. To achieve this, CPE was employed as a low cost, easy preparation and renewable electrode and hIL-2 oligonucleotide was utilized as the probe. Differential pulse voltammetry was used as an electrochemical method and the effect of some experimental factors was investigated. The specificity of the sensor was monitored using complementary and noncomplementary DNA chains for the hybridization event.

2. Experimental

2.1. Chemicals

Brilliant cresyl blue was of analytical grade and was purchased from Merk. A 20-mer oligonucleotide corresponding to antisense strand of human IL-2 gene (hIL-2) was used as the probe and its complementary (chIL-2) oligonucleotide corresponding to sense strand of human IL-2 was used as target DNA. 16SR, YF270 and HgbBF oligonucleotides were used as noncomplementary oligonucleotides. All of the oligonucleotides were supplied as lyophilized powder by MWG-Biotech Company, with the following sequences:

Probe DNA (hIL-2): 5′-GGA GGA AGT GCT AAA TTT AG-3′
Complementary DNA (chIL-2): 5′-CTA AAT TTA GCA CTT CCT CC-3′
Noncomplementary DNAs:
16SR: 5′-TAC CTT GTT AGG ACT TCA CC-3′
YF270: 5′-TGT AAA TTC TGT GAG TAT GAG-3′
HgbBF: 5′-TCA TTG AGT ACG GCT TGAC-3′.

The stock solutions of the oligonucleotides (100 μM) were prepared with TE buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More diluted solutions of the oligonucleotides were prepared using 0.50 M acetate buffer solution (pH 4.80) containing 20 mM NaCl. The stock solution of BCB (1 mM) was prepared using distilled and sterilized water. Other chemicals were of analytical grade. The distilled, deionized and sterilized water was used in all solution preparations. Each measurement consisted of the immobilization/detection cycle carried out on a fresh CPE surface. All the experiments were performed at room temperature in an electrochemical cell.

2.2. Apparatus

Electrochemical experiments were performed using AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.9 software package (Eco Chemie, Netherlands). The utilized three-electrode system was composed of a CPE (surface area of 0.015 cm^2) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode. A computing double beam UV spectrophotometer (CECIL 5000, elegant technology) with quartz cell was used to measure the absorbance.

2.3. Procedure

2.3.1. Preparation of the working electrode

The carbon paste electrode was prepared in the usual way by hand mixing graphite powder with paraffin oil in a ratio of 70:30 (w/w). A portion of the resulting paste was then inserted in the bottom of a glass tube. The electrical connection was implemented by a copper wire lead fitted into the glass tube. The surface of the resulting paste electrodes was smoothed on a weighing paper and rinsed carefully with distilled water.

2.3.2. Electrochemical activation of the CPE

Pretreatment of the polished electrode was done at optimized potential of +0.20 V vs. SCE for 5 min in 0.50 M acetate buffer solution (pH 4.80) containing 20 mM of NaCl without stirring. All experiments were done on activated CPE.

2.3.3. Immobilization of probe on the CPE

Following activation, the working electrode was immersed in 0.50 M acetate buffer solution (pH 4.80) containing 1 μM probe and 20 mM of NaCl by applying −0.50 V potential vs. SCE for 5 min into the stirred solution for immobilization of probe on the CPE. Then, the electrode was rinsed with sterilized and deionized water.

2.3.4. Hybridization

The hybridization was performed by immersing the probe modified CPE into a stirred hybridization solution (0.5 M acetate buffer pH 4.8) containing 1 μM of target oligonucleotide and 20 mM of NaCl, for 5 min, while the working electrode potential was held at +0.50 V vs. SCE. The electrode was washed with sterilized and deionized water to remove the non-hybridized DNA. For hybridization of probe with noncomplementary sequences, the same protocol was applied.

2.3.5. BCB accumulation on the CPE

Following immobilization of the single stranded DNA (ssDNA) probe on CPE, BCB was accumulated on the probe by dipping the electrode into BCB (1 mM) for 5 min with stirring and without applying any potential to the working electrode. Once BCB was accumulated, the electrode was rinsed with sterilized and deionized water. The same strategy was performed for the accumulation of BCB on the bare electrode and probe modified electrodes following hybridization of the probe with complementary or noncomplementary oligonucleotides.

2.3.6. Voltammetric measurements

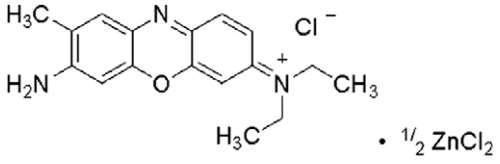
In cyclic voltammetric experiments, the electrode potential was scanned in −0.80 to +0.80 V vs. SCE in degassed BCB solution (1 mM) at scan rate of potential 100 mV s^{-1}. Electrochemical investigation was carried out using DPV in 0.1 M of phosphate buffer (pH 7.00) solution and scanning the electrode potential between −0.10 and −0.40 V vs. SCE at pulse amplitude of 50 mV.

The raw data were treated using the Savitzky and Golay filter (level 2) of GPES software, followed by the GPES software moving average baseline correction using a “peak width” of 0.01. Repetitive measurements were carried out following renewing the electrode surface by cutting and polishing of the electrode.

3. Results and discussion

3.1. Spectral characteristics

BCB is a cationic strong fluorescent dye and its structure is shown in Scheme 1. UV–Vis spectra of BCB, BCB-ssDNA and BCB-dsDNA are shown in Fig. 1. dsDNA was prepared by mixing equal amounts of two



Scheme 1. Structural formula of brilliant cresyl blue.