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Electrochemical detection of DNA hybridization by using a zirconia modified renewable carbon paste electrode

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ABSTRACT

A simple, polishable and renewable DNA biosensor was fabricated based on a zirconia modified carbon paste electrode. Zirconia was mixed with graphite powder and paraffin wax to produce the paste for the electrode, and response-optimized at 56% graphite powder, 19% ZrO_2 and 25% paraffin wax. An oligonucleotide probe with a terminal 5′-phosphate group was attached to the surface of the electrode via the strong affinity of zirconia for phosphate groups. DNA immobilization and hybridization were characterized by cyclic voltammetry and differential pulse voltammetry, using methylene blue as indicator. Examination of changes in response with complementary or non-complementary DNA sequences showed that the developed biosensor had a high selectivity and sensitivity towards hybridization detection ($\leq 2\times10^{-10}$ M complementary DNA detectable). The surface of the biosensor can be renewed quickly and reproducibly (signal RSD ± 4.6% for five successive renewals) by a simple polishing step.

1. Introduction

As an efficient method for detection of DNA hybridization, DNA biosensors hold an enormous potential for disease diagnosis, detection of infectious agents [1], drug screening and forensic applications [2–4]. Many efforts have been made to produce inexpensive, easy-to-use, fast-response devices to meet the challenges connected to the development of DNA biosensors. Electrochemical-based sensors offer sensitivity, selectivity and low cost for the detection of selected DNA sequences or mutated genes associated with human disease [5–9]. Electrochemical biosensors offer a powerful capacity for converting the hybridization event into an analytical signal, and consequently have been frequently proposed for detecting the DNA hybridization event [10].

Different kinds of solid electrodes, such as metal and carbon electrodes, have been employed as the transducers for electrochemical DNA biosensors [11,12]. Among these solid electrodes, carbon paste electrode has some specific advantages, including a wide potential window, low background current and easy fabrication [13,14]. Most importantly, the carbon paste electrode can be easily endowed with a specific property by introducing functional materials to the paste; moreover, the surface of electrode is polishable and renewable [15]. In the present work, zirconia is added to the paste due to its affinity for phosphate groups in the probe single-stranded DNA (ssDNA). The immobilization of probe DNA at the electrode is the key step in the preparation of DNA biosensors. Zirconia is an inorganic oxide with thermal stability, chemical inertness, lack of toxicity [16], and affinity for groups containing oxygen [17], so it is an ideal candidate as a material for immobilization of biomolecules with oxygen groups. Several methods, such as sol–gel [18] and electrodeposition [19], have been used to fabricate ZrO_2 modified electrodes for immobilization of DNA. Comparatively, the method based on a carbon paste electrode is simpler and more flexible. Methylene blue (MB), which is an organic dye that belongs to the phenothiazine family, is a well-known hybridization indicator of DNA due to its association with the free guanine bases of single-stranded DNA [20,21]. The affinity interaction between MB and ssDNA occurs quickly and generates a marked electrochemical signal. Thus, MB was applied as indicator in this paper.

In our work, we aimed to design a quick and effective method for detection of DNA hybridization. We have explored a new and available DNA biosensor based on a ZrO_2 modified electrode which could be fabricated with ease. No potential was applied to the electrode during process of probe ssDNA immobilization. Moreover, a renewable electrode could be achieved by incorporating a polishing procedure, which makes the biosensor more applicable.

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2. Materials and methods

2.1. Reagents and materials

The synthetic oligonucleotides were purchased from Shenggong Bioengineering (Shanghai, P. R. China). Their base sequences are as below:

Probe sequence: 5′-PO_4-GAG CGG CGC AAC ATT TCA GGT CGA-3′;
Complementary sequence: 5′-TCG ACC TGA AAT GTT GCG CCG CTC-3′;
Non-complementary sequence: 5′-GAG CGG CGC AAC ATT TCA GGT CGA TGG ACT AAC AGG TTA TTC GAA-3′.

Methylene blue (MB) and ZrO_2 were obtained from Sinopharm Chemical Reagent Co., Ltd. Paraffin wax and graphite powder were purchased from Shanghai Specimen Model Factory and Shanghai Colloid Chemical Plant respectively. Other reagents were commercially available and were all of analytical reagent grade, used without further purification.

All oligonucleotides stock solutions were prepared using TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0); 0.1 M phosphate buffer (pH 6.9) was used also. All solutions were prepared with doubly distilled water.

2.2. Apparatus and electrodes

Electrochemical experiments were carried out on a CHI440A microcomputer-based electrochemical workstation (CHI Instrument Inc. USA) with a conventional three-electrode system. A saturated calomel electrode (SCE) was used as reference electrode and a platinum wire as counter electrode. The CPE with or without ZrO_2 was used as the working electrode.

2.3. General procedures

2.3.1. Construction of CPEs

Paraffin wax (0.2 g) was heated to melting under an infrared lamp and then was mixed with graphite powder (0.45 g) and ZrO_2 (0.15 g) to produce a homogenous paste. The ZrO_2 modified carbon paste electrode (denoted as ZrO_2-CPE) was prepared by firmly packing the paste into an electrode cavity (2 mm diameter) of a polytetrafluorethylene sleeve. Electrical contact was established by a copper wire. The CPE without ZrO_2 was prepared in a similar way using only graphite powder and paraffin wax. The surfaces of both modified and unmodified CPEs were carefully smoothed on weighing paper and rinsed with doubly distilled water thoroughly.

2.3.2. Immobilization of ssDNA probe on CPEs and hybridization

The immobilization of the ssDNA probe on the electrode surface was carried out as follows: a freshly prepared ZrO_2-CPE was immersed in 2.0 mL TE buffer containing 2.25×10^{-7} M probe ssDNA for 60 min at room temperature (25 ± 0.5 °C). The electrode was subsequently rinsed three times with a 0.1% sodium dodecyl sulfate (SDS) phosphate buffer (pH 6.9) to remove any unimmobilized ssDNA. For comparison, the CPE without ZrO_2 was treated in the same way as above.

The hybridization was performed at room temperature by dipping the probe-modified electrode into 2.0 mL TE buffer containing 2.25×10^{-7} M complementary ssDNA for 30 min. The electrode was washed three times with a 0.1% sodium dodecyl sulfate (SDS) phosphate buffer (pH 6.9) to remove any unhybridized DNA. Another probe-modified electrode was exposed to 2.0 mL TE buffer containing 2.25×10^{-7} M non-complementary ssDNA and was treated using the same method.

2.3.3. Electrochemical detection

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used in the electrochemical measurement. The parameters employed for CV and DPV were as follows: CV, potential range −0.35 to 0 V, scan rate 50 mV/s; DPV, potential 0 to −0.5 V, pulse amplitude 50 mV, pulse width 50 ms, pulse period 0.2 s. CV measurements were performed in 0.1 M phosphate buffer solution (pH 6.9) containing 2×10^{-5} M MB. TE buffer was used as the supporting electrolyte for DPV experiments; all electrodes used for DPV experiments were immersed in TE buffer containing 2×10^{-5} M MB for 10 min before measurement.

3. Results and discussion

3.1. Electrode optimization

Electrode optimization was performed with respect to the fabrication feasibility and response current intensity of the modified electrode. In order to avoid making the CPE too friable, 25% (w/w) paraffin wax was used in the carbon paste in all cases. The content of graphite powder in the carbon paste was varied from 37.5% to 62.5% (w/w); ZrO_2 formed the remainder. For each electrode, cyclic voltammetry was carried out in 0.1 M phosphate buffer solution (pH 6.9) containing 2×10^{-5} M MB. The reduction peak currents were taken as a criterion to assess the electrode performance. Fig. 1 shows that the peak current increases when more graphite powder was added to the paste until the content of graphite reaches 56%; further increase in the percentage of graphite powder contributes to the decrease in the peak current, presumably because the ZrO_2 level is becoming critical. Enough ZrO_2 is necessary for efficient immobilization of the ssDNA probe. Consequently, in our experiment the optimized paste for CPE contains 56% graphite powder, 19% ZrO_2 and 25% paraffin wax.

3.2. Preliminary investigation

The cyclic voltammograms of MB in 0.1 M phosphate buffer (pH 6.9) at different kinds of CPEs, including unmodified CPE, ZrO_2-CPE and probe ssDNA immobilization ZrO_2-CPE (denoted as ssDNA/ZrO_2-CPE), are shown in Fig. 2, from which we can see that the peak current decreases in the order ssDNA/ZrO_2-CPE (curve c)>unmodified CPE (curve b)≥ZrO_2-CPE (curve a). As the ZrO_2-CPE electrode has almost the same peak current as the unmodified CPE, this indicates that the introduction of ZrO_2 to the CPE does not influence the response current markedly and ZrO_2 exhibits no special absorption of MB. The peak current of ssDNA/ZrO_2-CPE is significantly more than the ZrO_2-CPE; this reflects the successful immobilization of probe ssDNA on the ssDNA/ZrO_2-CPE, through attachment of ZrO_2 with the 5′-phosphate group of probe ssDNA.

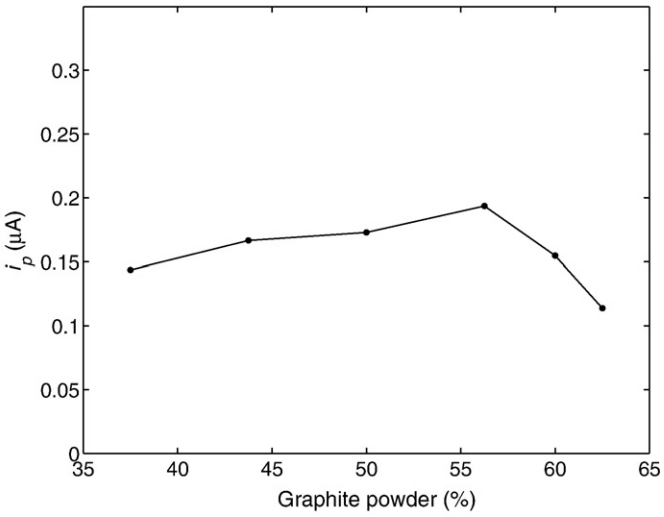

Fig. 1. Reduction peak current of modified CPE in 0.1 M phosphate buffer solution (pH 6.9) containing 2×10^{-5} M MB as a function of percent graphite powder in a carbon paste.

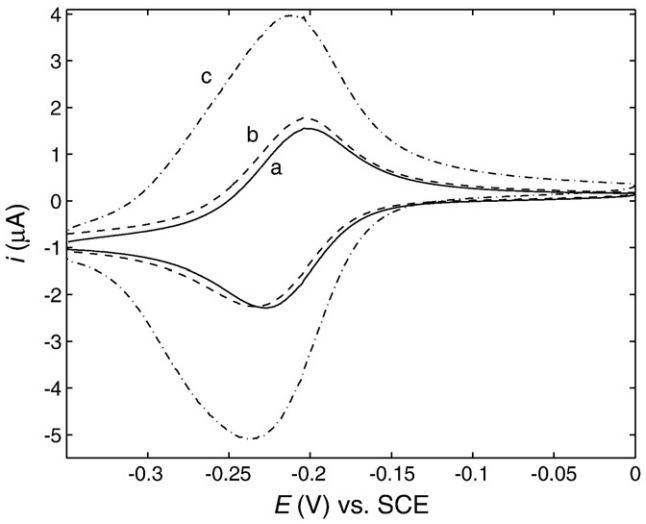

Fig. 2. Cyclic voltammograms of (a) ZrO_2 modified CPE (ZrO_2-CPE), (b) unmodified CPE, (c) ssDNA/ZrO_2-CPE, all in 0.1 M phosphate buffer solution (pH 6.9) containing 2×10^{-5} M MB at scan rate of 50 mV/s.

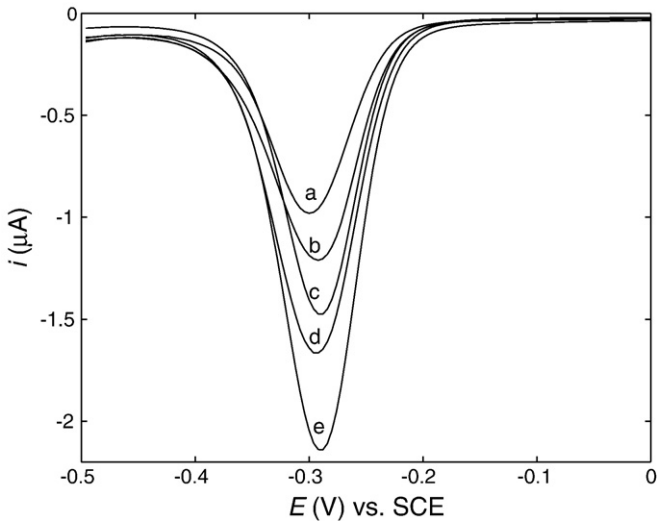

Fig. 4. Differential pulse voltammogramms using 2.0×10^{-5} M MB as the redox indicator for different target concentrations: (a) 2.25×10^{-7} M, (b) 2.25×10^{-8} M, (c) 2.25×10^{-9} M, (d) 2.25×10^{-10} M, (e) 0 M.

3.3. Electrochemical detection of DNA hybridization with the DNA sensor

DNA hybridization was detected by means of differential pulse voltammetry (DPV). Differential pulse voltammetric responses of MB accumulated on several different electrodes are recorded in Fig. 3. Typically, there is a low MB reduction signal (curve a, b) on the bare unmodified CPE and on the ZrO_2-CPE due to low accumulation of MB on the bare electrodes. The largest MB reduction signal (curve e) was observed with the ssDNA/ZrO_2-CPE because MB has a strong affinity for free guanine bases present in ssDNA and hence the greatest amount of MB accumulated on the electrode surface. However, a significant decrease in the signal of MB (curve c) was observed following hybridization of complementary target ssDNA with the ssDNA/ZrO_2-CPE. This may be attributed to less MB accumulation on the double-stranded DNA (dsDNA) caused by guanine bases becoming inaccessible to MB with hybridization. This result indicates the occurrence of hybridization of the target complementary ssDNA with the probe-modified CPE.

The selectivity of this assay was investigated by using the ssDNA/ZrO_2-CPE probe to examine any interaction with the uncomplimentary ssDNA. The DPV signal of MB with this electrode was also recorded (Fig. 3, curve d), and shows a very similar signal to that recorded with ssDNA/ZrO_2-CPE alone; this reflects the expected absence of hybridization interaction between ssDNA/ZrO_2-CPE and uncomplimentary ssDNA. The results demonstrate that only a complementary sequence could form a dsDNA with the DNA probe and give a significant decrease in DPV signal. A negligible change in the signal observed for a non-complementary sequence shows the high selectivity of the hybridization detection.

The sensitivity of the electrochemical hybridization assay was investigated by varying the target ssDNA concentration. Different peak currents obtained in the DPV response of MB after hybridization of the probe with the target were recorded (Fig. 4). As Fig. 5 shows, the reduction current of MB was linear with respect to the logarithmic value of the complementary ssDNA concentration over the range from

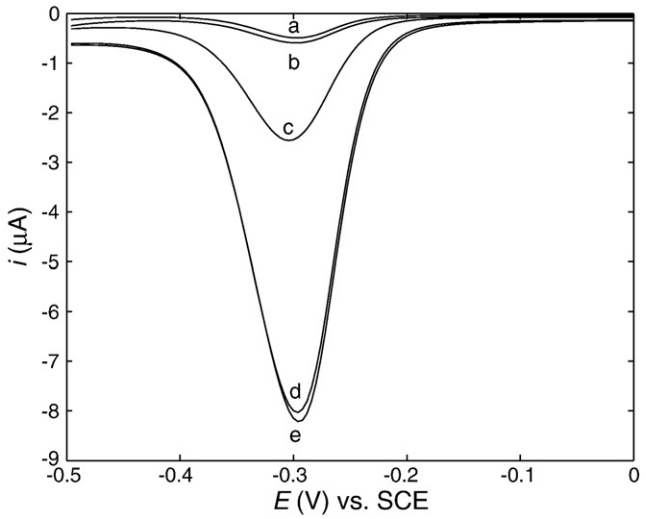

Fig. 3. Differential pulse voltammograms using 2.0×10^{-5} M MB as the redox indicator for (a) ZrO_2-CPE unmodified by probe ssDNA, (b) unmodified CPE after immersed in TE solution containing probe ssDNA, (c) ssDNA/ZrO_2-CPE after hybridization with the complementary oligonucleotide sequence (2.25×10^{-7} M), (d) ssDNA/ZrO_2-CPE after exposure to the non-complementary oligonucleotide sequence (2.25×10^{-7} M), (e) ssDNA/ZrO_2-CPE alone.

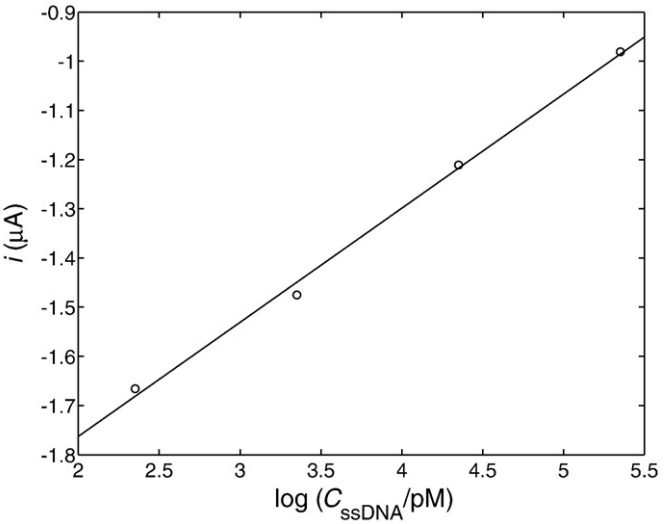

Fig. 5. Relationship between log[target oligonucleotide concentration] and peak current. The regression equation of the calibration line is: $I_{peak\ current}=0.2319\times\log(C_{ssDNA}/pM)-2.227$, $R^2=0.9965$.

2.25×10^{-10} to 2.25×10^{-7} M. The regression equation was $I_{\text{peak current}} = 0.2319\times\log(C_{\text{ssDNA}}/\text{pM}) - 2.227$, ($I_{\text{peak current}}$ was the DPV peak current of MB, μA; C_{ssDNA} was the concentration of target complementary ssDNA, pM), and the regression correlation coefficient (R^2) of the calibration line was 0.9965.

3.4. Renewability investigation

The surface of the biosensor could be renewed by polishing on a weighing paper, followed by the usual probe ssDNA immobilization procedure being performed on the renewed electrode. The DPV response was examined under the same condition described above. The relative standard deviation was 4.6% for five successive renewals. Thus, the renewable electrodes have good reproducibility.

4. Conclusions

This paper describes a polishable and renewable DNA sensor for detection of DNA hybridization based on a ZrO_2 modified carbon paste electrode. During the whole fabrication process of the sensor, only several simple procedures were performed and no applied potential or any other electrochemical method was needed. High sensitivity and selectivity were achieved by DPV experiments with MB as the indicator. Furthermore, the sensor could be renewed easily and reproducibly by a simple and quick polishing step. The results show that this method is a simple and practical method with good selectivity for quantitative detection of DNA hybridization. This new methodology may be applicable in a wide variety of biosensing applications.

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