

Electrochemical DNA Biosensor Based on Graphene and TiO₂ Nanorods Composite Film for the Detection of Transgenic Soybean Gene Sequence of MON89788

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Abstract

Based on graphene (GR), TiO₂ nanorods, and chitosan (CTS) nanocomposite modified carbon ionic liquid electrode (CILE) as substrate electrode, a new electrochemical DNA biosensor was effectively fabricated for the detection of the transgenic soybean sequence of MON89788. By using methylene blue (MB) as hybridization indicator for monitoring the hybridization with different ssDNA sequences, the differential pulse voltammetric response of MB on DNA modified electrodes were recorded and compared. Due to the synergistic effects of TiO₂ nanorods and GR on the electrode surface, the electrochemical responses of MB were greatly increased. Under optimal conditions the differential pulse voltammetric response of the target ssDNA sequence could be detected in the range from 1.0×10^{-12} to 1.0×10^{-6} mol/L with a detection limit of 7.21×10^{-13} mol/L (3σ). This electrochemical DNA biosensor was further applied to the polymerase chain reaction (PCR) product of transgenic soybeans with satisfactory results.

Keywords: TiO₂ nanorods, Graphene, Chitosan, Carbon ionic liquid electrode, Electrochemical DNA biosensor

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1 Introduction

Electrochemical DNA sensors offer high sensitivity, good selectivity and low cost for the detection of selected DNA sequences in very small sample volumes, which has arisen great interests in the field of DNA detection [1,2]. Hashimoto et al. fabricated different kinds of electrochemical DNA sensors for the detection of specific gene sequences with electrochemically active dyes [3–5]. The performances of the electrochemical DNA sensor can be greatly influenced by the immobilization of probe ssDNA sequences on the electrode surface. Different kinds of methods such as covalent binding, adsorption and polymerization have been devised for the probe ssDNA immobilization. In recent years various kinds of nanoparticles have been utilized for the probe ssDNA immobilization due to their unique characteristics such as high surface area, excellent biocompatibility, good conductivity and strong adsorption ability [6]. Zhang et al. [7] fabricated an electrochemical DNA sensor based nano-ZnO/multiwalled carbon nanotubes/chitosan nanocomposite membrane. Selvaraju et al. [8] fabricated a sandwich-type electrochemical DNA biosensor using streptavidin-coated magnetic beads and gold nanoparticles as response magnifiers. Li et al. [9] reported an electrochemical DNA biosensor with 4-aminothiophenol self-assembled on an electrodeposited nanogold electrode coupled with Au nanoparticles. Gao et al. [10] reported an electrochemical de-

oxyribonucleic acid biosensor based on a self-assembly film with nanogold decorated on ionic liquid modified carbon paste electrode. By using different kinds of modifiers on the electrode surface, the ssDNA sequence can be fixed on the electrode with various orientations, which influence the interaction model of electrochemical indicator with DNA sequence. Some preparation procedures for the electrochemical DNA sensor are too complicated with several steps involved, other reports use expensive reagents, labeled DNA sequence or specific nanomaterials that are not commercially available, which limit the practical application in real samples detection.

As a single layer of carbon atom in a closely packed honeycomb two-dimensional lattice, graphene (GR) can greatly promote the electron transfer rate and electrocatalytic activity owing to its unique properties such as large specific surface area and high mobility of charge carriers [11]. The unique two dimensional crystal structure makes it as extremely attractive supporting material for the incorporation of biomolecules and nanoparticles [12]. GR has exhibited many advantages such as wide potential windows, relatively inert electrochemistry, excellent electrocatalytic activities with favorable microenvironment, which could be used for the fabrication of electrochemical biosensor. A number of electrochemical DNA biosensors based on GR and nanocomposite have been reported in recent years. Lim et al. [13] applied the epitaxial GR as an anode material for the simultaneous

detection of four DNA bases in double-stranded DNA. Zhao et al. [14] developed an electrochemical DNA sensor based on GR quantum dots modified pyrolytic graphite electrode coupled with probe ssDNA sequences. Sun et al. [15] reported an electrochemical DNA sensor based chitosan/Fe₃O₄ microsphere-GR composite modified electrode.

As a kind of semiconductor, titanium dioxide (TiO₂) nanomaterials have been used in the field of electrochemical biosensors with properties such as large surface area, thermal stability and good biocompatibility. Liu et al. [16] used TiO₂ nanoparticles modified glassy carbon electrode (GCE) for the selective detection of dopamine in the presence of ascorbate and uric acid. TiO₂ nanoparticles can also be used for the immobilization of protein to realize the direct electrochemistry [17,18]. Recently TiO₂ nanoparticles decorated GR nanocomposite has been reported, which combined the advantages of TiO₂ and GR with better electrochemical performances. Wang et al. [19] reported that TiO₂-GR nanocomposite could remarkably improve the Li-ion insertion/extraction property and specific capacity of Li-ion battery. Fan et al. [20] applied a TiO₂-GR nanocomposite modified electrode for the detection of adenine and guanine. Fan et al. [21] detected L-tryptophan and L-tyrosine using a GCE modified with a Nafion/TiO₂-GR composite film. The presence of TiO₂-GR nanocomposite on the electrode surface exhibited excellent performance due to the good adsorptivity and conductivity.

Due to the high ionic conductivity and wide electrochemical potential window [22], ionic liquids (ILs) have been widely utilized in the field of electrochemistry and electrochemical sensors. One of the approaches is devoted to fabricate IL-based modified electrodes with different techniques including direct mixing [23,24], casting and rubbing [25], electrodeposition [26], layer-by-layer [27] and so on. Carbon ionic liquid electrode (CILE), which is prepared by using IL as binder and/or modifier in a carbon paste electrode, has been proved to exhibit larger potential window in aqueous systems with an improved current density in comparison with other commonly used carbon electrode [23]. CILE has been shown to be an attractive and efficient working electrode to fabricate different kinds of electrochemical sensors and biosensors [28].

Soybean event MON89788 is a second generation glyphosate-tolerant soybean product. In addition to providing flexibility, simplicity and cost-effective weed control options, MON89788 and varieties contain the trait that have the potential to enhance yield and thereby further benefit farmers and the soybean industry. In this paper, a 1-butylpyridinium hexafluorophosphate (BPPF₆) based CILE was used as substrate electrode and further modified with the nanocomposite composed of chitosan (CTS)-GR-TiO₂ nanorods to fabricate an electrochemical DNA biosensor. CTS is a biopolymer that is commonly used in electrochemical sensor due to its biocompatibility and good film forming ability, which could fix the nano-

materials tightly on the electrode surface. Due to the presence of large quantities of amine groups on the molecular chains, CTS can adsorb the negatively charged probe ssDNA sequences. The synergistic effects of TiO₂ nanorods and GR nanosheets can increase the adsorption amounts of probe ssDNA sequences on the electrode surface and result in the corresponding increase of the electrochemical responses of MB. The new electrochemical DNA biosensor was successfully applied to the detection of transgenic soybean MON89788 specific ssDNA sequences with advantages such as the simpler preparation procedure, higher selectivity and broader linear range.

2 Experimental

2.1 Apparatus

A CHI 750B electrochemical workstation (Shanghai Chenhua Instrument, China) was used for all the electrochemical measurements including cyclic voltammetry, electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). The traditional three electrode system was used with a modified CILE as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. The morphologies of nanomaterials were obtained on a JSM-7500F scanning electron microscope (Japan Electron Company, Japan).

2.2 Reagents

1-Butylpyridinium hexafluorophosphate (BPPF₆, >99%, Lanzhou Greenchem. ILS. LIPC. CAS., China), graphite powder (average particle size 30 µm, Shanghai Colloid Chemical Company, China), chitosan (CTS, Dalian Xindie Limited Company, China) and methylene blue (MB, Shanghai Chemicals Plant, China) were used as received. GR was synthesized according to the modified hummer's method [29,30] and TiO₂ nanorods were synthesized according to the reported procedure with minor modification [31]. Different kinds of buffers such as 50.0 mmol/L PBS (pH 7.0) and 50.0 mmol/L Tris-HCl buffer (pH 7.0) were used. All the solutions were prepared with doubly distilled water and other chemicals used were of analytical grade.

The 21-base oligonucleotides probe ssDNA sequence, target ssDNA sequence, one-base mismatched ssDNA sequence and three-base mismatched ssDNA sequence, which were related to the soybean transgenic sequence of MON89788, were synthesized by Shanghai Sangon Biological Engineering Tech. Co. Ltd. (China). Their base sequences were as below:

probe ssDNA sequence:

5'-CTG AAG GCG GGA AAC GAC AAT-3',

target ssDNA sequence:

5'-ATT GTC GTT TCC CGC CTT CAG-3',

one-base mismatched ssDNA sequence:

5'-ATT GTC GTT TCA CGC CTT CAG-3',

three-base mismatched ssDNA sequence:

5'-ATC GTC GTT TCA CGC CTT AAG-3',

The DNA sample for polymerase chain reaction (PCR) amplification was extracted from soybean. The PCR reaction was performed on an Eppendorf Mastercycler Gradient PCR system using oligonucleotide primers for soybean MON89788 gene with the following sequences:

Primer F: 5'-TCC CGC TCT AGC GCT TCA AT-3';

Primer R: 5'-TCG AGC AGG ACC TGC AGA A-3'.

2.3 Fabrication of Modified Electrodes

CILE was fabricated with the following procedure [32]. Initially, 3.0 g of graphite powder and 1.0 g of BPPF₆ were mixed thoroughly in a mortar and further heated to 80 °C to form a homogeneous carbon paste. A portion of the carbon paste was filled into one end of a glass tube ($\varnothing=4$ mm) and a copper wire was inserted through the opposite end to establish an electrical contact. Prior to use a mirror-like surface was obtained by polishing the electrode on a weighing paper.

The nanocomposite was prepared by the addition of 0.5 mg TiO₂ nanorods and 1.0 mg GR into 1.0 mL 1.0 mg/mL CTS solution and sonicated for forming a homogeneous mixture of CTS-GR-TiO₂. Then 10 μ L of the nanocomposite was cast on the CILE surface and dried at the room temperature to get the modified electrode denoted as CTS-GR-TiO₂/CILE. Other modified electrodes such as CTS/CILE, CTS-TiO₂/CILE and CTS-GR/CILE were prepared by casting 10 μ L of 1.0 mg/mL CTS that contained 0.5 mg/mL TiO₂ nanorods or 1.0 mg/mL GR on CILE surface to get the modified electrodes for comparison.

2.4 Immobilization of Probe ssDNA on CTS-GR-TiO₂/CILE

Immobilization of probe ssDNA sequence was achieved by dropping 10.0 μ L of 1.0×10^{-6} mol/L probe ssDNA sequence (in pH 7.0 PBS) directly onto the surface of CTS-GR-TiO₂/CILE. CTS is a cationic polymer with abundant -NH₂ group, which was in positive charged. While the phosphate skeleton of probe ssDNA was in negative charged, so the probe ssDNA could be immobilized on the surface of CTS-GR-TiO₂/CILE through electrostatic attraction between probe ssDNA and CTS film with most of the ssDNA laid down on the electrode surface [33]. After drying in air at room temperature, the electrode surface was washed with 0.5% SDS solution and double distilled water to remove unadsorbed probe ssDNA sequence and the resulted electrode was denoted as ssDNA/CTS-GR-TiO₂/CILE.

The hybridization assay was carried out by dropping 10.0 μ L different concentrations of target ssDNA sequence onto the surface of ssDNA/CTS-GR-TiO₂/CILE for 4 h at room temperature. Then the electrode was washed with 0.5% SDS solution and double distilled water to remove the unhybridized target ssDNA and this

hybridized electrode was denoted as dsDNA/CTS-GR-TiO₂/CILE. The similar procedure was also performed with other kinds of ssDNA sequence to test the selectivity of hybridization.

2.5 Electrochemical Detection

MB was accumulated on the hybrid surface by immersing the modified electrode into 5.0 mL of 2.0×10^{-5} mol/L MB solution for 8 min. After the accumulation the electrode was washed with double distilled water for three times and the modified electrode was transferred into a 50.0 mmol/L Tris-HCl buffer solution (pH 7.0) for the voltammetric measurement by DPV method with the instrumental parameters set as: pulse amplitude 0.001 V, pulse width 0.05 s and pulse period 0.2 s.

2.6 Polymerase Chain Reaction Procedure

The soybean samples were added into 10 mL concentrated digestion solution and kept 48 h in water bath at 25 °C. Then the solution was centrifuged 4 minutes at 12000 rpm and obtained the precipitate, which was dispersed in 100 μ L sterilized distilled water. Immediately the DNA was extracted using DNA extraction kit (Beijing Tiangen Biotech. Co. Ltd. China).

The amplification of MON89788 gene was performed by adding 200.0 nmol/L primer F and primer R of MON89788 gene sequence, 10 $\times$ reaction buffer B (Promega, Wisconsin USA), 2.0 mmol/L MgCl₂, 200.0 nmol/L each of dATP, dCTP, dGTP and dTTP, 1.5 units of Taq DNA polymerase (Promega, Wisconsin USA), 1.0 μ L DNA template purified from soybean samples in 0.2 mL reaction tube and the final volume was 25 μ L. PCR conditions were optimized as follows: 35 cycles of amplification (94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s) and final extension at 72 °C for 5 min. 6 μ L each of the PCR products were analyzed by electrophoresis separation (5 V/cm, 40 min) on a 2% agarose gel containing 0.5 μ g/mL of ethidium bromide in 1 $\times$ TAE buffer (40.0 mmol/L Tris, 1.0 mmol/L EDTA, 40.0 mmol/L acetate, pH 8.0). The obtained PCR products of MON89788 gene were kept at 4 °C before use.

3 Results and Discussion

3.1 SEM Image of the Materials

SEM images of the materials used such as GR nanosheets, TiO₂ nanorods and GR-TiO₂ nanocomposite were recorded and shown in Figure 1. It can be seen that GR showed clearly large sheet-like shape with slightly scrolled edges (Figure 1A), and most GR nanosheets were lying flat with some GR nanosheets fold together. The synthesized TiO₂ nanomaterials appeared as longer nanorods with uniform diameter about 50 nm with smooth surface (Figure 1B). Figure 1C exhibited the SEM image of GR-TiO₂ nanocomposite. It can be seen that TiO₂ nanorods were well dispersed on the surface of GR and some

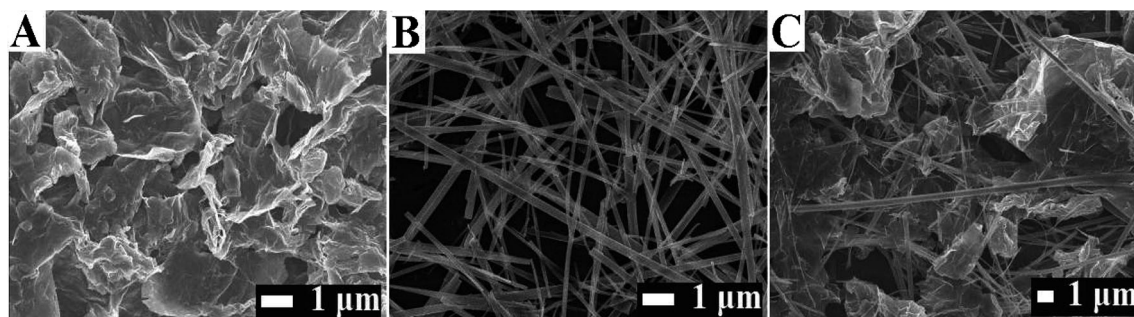


Fig. 1. SEM images of GR (A), TiO₂ nanorods (B) and GR-TiO₂ nanocomposite (C).

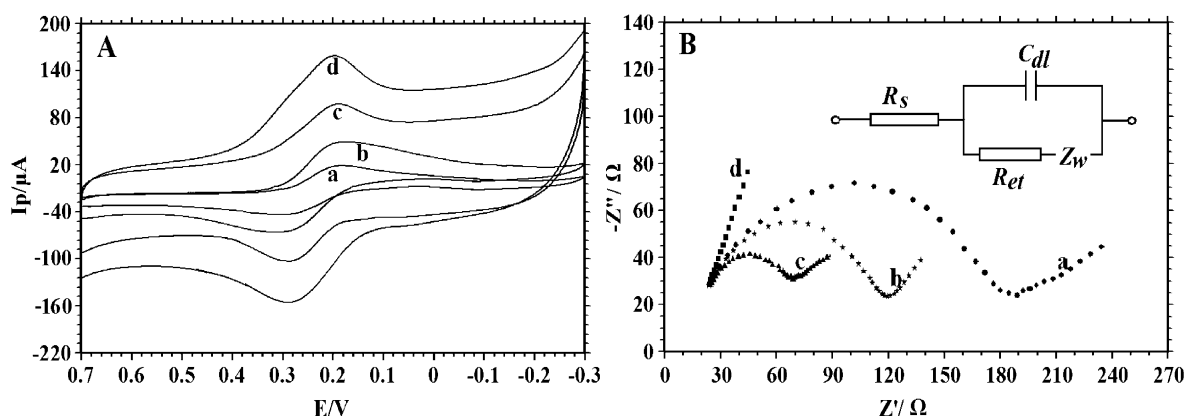


Fig. 2. (A) Cyclic voltammograms of different modified electrodes in 1.0 mmol/L K₃[Fe(CN)₆] and 0.5 mol/L KCl with scan rate of 100 mV/s. (B) Electrochemical impedance spectra of different modified electrodes in 1.0 mmol/L [Fe(CN)₆]^{3-/4-} containing 0.1 mol/L KCl with the frequency sweep from 10⁴ to 1.0 Hz. Electrodes used (from a to d) were CTS/CILE, CTS-TiO₂/CILE, CTS-GR/CILE and CTS-GR-TiO₂/CILE, respectively. Inset: Randles equivalent circuit used to model impedance data in the presence of redox couples.

of them inserted into the sheets structure of GR, indicating the completely and uniform mix of TiO₂ nanorods with GR to get a homogeneously composite.

3.2 Electrochemical Behavior of the Modified Electrodes

Figure 2A showed the cyclic voltammograms of CTS/CILE (a), CTS-TiO₂/CILE (b), CTS-GR/CILE (c) and CTS-GR-TiO₂/CILE (d) obtained in a mixture solution of 1.0 mmol/L [Fe(CN)₆]³⁻ and 0.5 mol/L KCl. Also the composition of the modifier and the electrochemical data of different modified electrodes were summarized in Table 1. At a scan rate of 100 mV/s quasi-reversible redox peaks of K₃[Fe(CN)₆] were observed on CTS/CILE with a peak-to-peak separation (ΔE_p) of 95 mV and the smallest redox peak currents (curve a). When the electrode

was modified with TiO₂, the redox peak currents of CTS-TiO₂/CILE (curve b) increased slightly with a ΔE_p value of 117 mV, which was due to the presence of semiconductive TiO₂ nanorods on the electrode surface. Due to the large surface area of TiO₂ nanorods, the redox currents increased. At the same the ΔE_p value also increased, which was attributed to the poor conductivity of TiO₂ nanorods. While on CTS-GR/CILE (curve c), the redox peak currents increased greatly with a ΔE_p value of 71 mV, which was ascribed to the presence of the high conductive GR nanosheets on the electrode surface with increased surface area and excellent electroconductivity. Also the conductivity of GR was higher than that of the semiconductive TiO₂ nanorods, so the redox peak currents on CTS-GR/CILE were bigger than that of CTS-TiO₂/CILE. On CTS-GR-TiO₂/CILE the biggest redox

Table 1. The composition and the electrochemical data of different modified electrodes.

Electrode	Modifier	I_{pc} (μA)	I_{pa} (μA)	ΔE_p (mV)	Observed capacitance (μF/cm ²)
CTS/CILE	1.0 mg/mL CTS	29.2	28.6	95	4.48
CTS-TiO ₂ /CILE	1.0 mg/mL CTS + 0.5 mg/mL TiO ₂	35.7	37.8	117	7.72
CTS-GR/CILE	1.0 mg/mL CTS + 1.0 mg/mL GR	52.4	57.2	71	18.58
CTS-GR-TiO ₂ /CILE	1.0 mg/mL CTS + 1.0 mg/mL GR + 0.5 mg/mL TiO ₂	82.5	84.1	65	27.31

peaks appeared (curve d) with a ΔE_p value decreased to 65 mV, which was more close to the ideal value of 59 mV for the ferricyanide system. The results indicated the presence of GR-TiO₂ nanocomposite could further increase the electron transfer rate due to its synergistic effects with bigger surface area and better conductivity. The detailed electrochemical data on different modified electrodes were listed in Table 1. The increase of redox peak current and the decrease of peak-to-peak separation with step-by-step modification of the electrode indicated that the CTS-GR-TiO₂ nanocomposite on the electrode surface exhibited best performance.

EIS can be used to probe the interfacial electron transfer resistance (R_{et}) on the modified electrodes and the Randles equivalent circuit is used to fit the impedance spectra (inset of Figure 2B). The circuit includes the ohmic resistance of the electrolyte (R_s), the Warburg impedance (Z_w) that results from the ions in electrolytic solution to electrode, the interfacial electron transfer resistance (R_{et}), and the interface double layer capacitance (C_{dl}). The two component R_{et} and C_{dl} depend on the dielectric and insulating feature at the electrode interface. In the Randles circuit it is assumed that R_{et} and Z_w are both parallel to the C_{dl} . This parallel combination of R_{et} and C_{dl} give rise to the semicircle in the Randle circuit [34]. In these results the semicircle portion observed at high frequencies in the Nyquist diagrams corresponds to the electron transfer limiting process and the linear part at lower frequencies corresponds to the diffusion-limited process. The Nyquist diagrams of different modified electrodes in 1.0 mmol/L [Fe(CN)₆]^{3-/4-} and 0.1 mol/L KCl solution were recorded sweeping the frequencies from 10⁴ to 1.0 Hz and the results were shown in Figure 2B. The R_{et} value that was derived from the semicircle diameter of the impedance spectra was equal to 160.5 Ω for CTS/CILE (curve a). After the modification of the CILE surface with TiO₂ nanorods or GR, R_{et} values of CTS-TiO₂/CILE and CTS-GR/CILE of 89.7 Ω (curve b) and 42.3 Ω (curve c) were obtained, respectively. The results also indicated the higher conductivity of GR nanosheets than TiO₂ nanorods on the electrode surface. While a straight line appeared on CTS-GR-TiO₂/CILE (curve d) when TiO₂ nanorods and GR were present on the electrode surface simultaneously, which indicated the nanocomposite could effectively accelerate the electron transfer rate. The EIS results were also in good agreement with the cyclic voltammetric responses of [Fe(CN)₆]^{3-/4-} on the different modified electrodes, indicating the successful immobilization of the nanocomposite on the electrode surface.

The modified electrodes were immersed into 0.05 mmol/L pH 7.4 PBS and scanned between 0.0 and 0.6 V at different scan rates from 10 to 500 mV/s. Then the observed capacitance of different modified electrodes were further calculated based on the following equation [35]: $C_{app} = I/Sv$ (I is the average of the positive and negative charging current obtained at 0.4 V, S is the geometric area of the electrodes and v is the scan rate). By exploring the slope of the straight line of I versus v , the ob-

served capacitance of different modified electrodes were calculated with the results listed in Table 1. It can be seen that the capacitances increased with step-by-step modification of the nanomaterials, which indicated that a composite film was formed on the electrode surface with increasing layered structure and large surface area, resulting in the increase of double layer at the electrode/electrolyte interface.

3.3 Electrochemical Behavior of MB on the Probe ssDNA Modified Electrode

MB is a phenothiazine dye that is well-known to be used as indicator in the development of an electrochemical DNA biosensor due to its high affinity with the guanine bases on DNA molecules [36]. Differential pulse voltammograms of the cathodic signals of MB on different modified electrodes such as ssDNA/CTS/CILE (curve a), ssDNA/CTS-TiO₂/CILE (curve b), ssDNA/CTS-GR/CILE (curve c) and ssDNA/CTS-GR-TiO₂/CILE (curve d) were recorded in 50.0 mmol/L pH 7.0 Tris-HCl buffer solution. As can be seen in Figure 3, the reduction peak current of MB showed a corresponding increase with the forward modification of the nanomaterials on the electrode surface, which indicated the increase of the probe ssDNA amount on the modified electrode. The increase of ssDNA on the electrode surface can result in the increase of the MB molecules accumulated on the electrode surface, and then the increase of the reduction peak current. The increase of reduction peak currents of MB on the different modified electrodes also prove the presence of TiO₂ nanorods and GR nanosheets on the electrode surface could increase the effective surface area of the electrode interface with the increase of the probe ssDNA immobilization amount. The highest reduction current that appeared at -0.293 V on ssDNA/CTS-GR-TiO₂/CILE (curve d) indicated the maximum absorption amounts of probe ssDNA sequence on the electrode surface, which provided more binding sites for MB simulta-

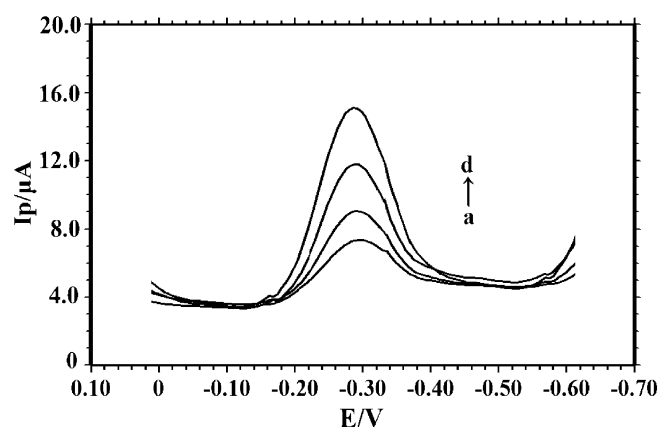


Fig. 3. Differential pulse voltammograms of (a) ssDNA/CTS/CILE, (b) ssDNA/CTS-TiO₂/CILE, (c) ssDNA/CTS-GR/CILE and (d) ssDNA/CTS-GR-TiO₂/CILE using MB as the indicator in 50.0 mmol/L pH 7.0 Tris-HCl buffer solution.

neously. CTS is a kind of natural cationic polymer which is often used in electrochemical DNA sensors for the immobilization of ssDNA through electrostatic interactions. The addition of GR and TiO_2 nanorods in the CTS film could effectively further increase the surface-to-volume ratio with large surface area and adsorb more ssDNA. Meanwhile the good conductivity of GR nanosheets could accelerate the electron transfer rate between the redox-active MB molecules and the electrode surface. So the biggest electrochemical response of MB was found on ssDNA/CTS-GR- TiO_2 /CILE.

3.4 Optimization of Conditions

The composition of the nanocomposite could influence the electron transfer rate of MB and the maximum loading amount of probe ssDNA on the electrode surface. So composites with different ratios of GR nanosheets and TiO_2 nanorods, such as 1:0.1, 1:0.3, 1:0.5, 1:0.7, 1:0.9, were studied. When the quantity of semiconductive TiO_2 nanorods was too high, the conductivity of the nanocomposite membrane modified electrode decreased obviously with smaller reductive peak current. When 1.0 mg GR and 0.5 mg TiO_2 were added to the 1.0 mL CTS solution, the reduction current of MB was maximum. Thus this ratio (1:0.5) was chosen to fabricate the modified electrode.

The influences of MB concentration and accumulation time used in the electrochemical indication on the reductive response of MB were investigated. Figure 4A demonstrates the relationship of the reduction current with MB concentration by immersing ssDNA/CTS-GR- TiO_2 /CILE into different MB solutions. It was clear that the DPV signals increased to a maximum value when the concentration of MB was 20.0 $\mu\text{mol/L}$ and then leveled off. When the accumulation time increased from 2 to 8 min the reduction current of MB was increased gradually and reached the maximum value at 8 min without further changes (Figure 4B). So the optimal conditions were selected as 20.0 $\mu\text{mol/L}$ MB and 8 min for accumulation.

3.5 Selectivity of the Electrochemical DNA Biosensor

The selectivity of this DNA biosensor was investigated by using the probe ssDNA modified electrode to hybridize

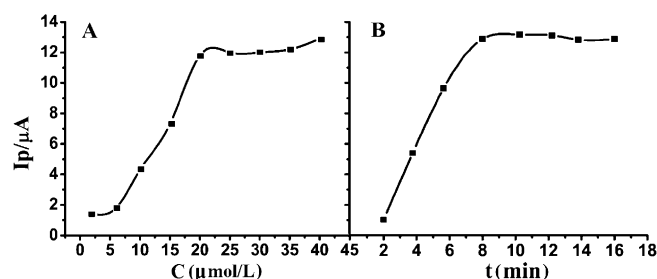


Fig. 4. Influences of MB concentration (A) and accumulation time (B) on the electrochemical response of MB on ssDNA/CTS-GR- TiO_2 /CILE.

with different ssDNA sequences related to the target ssDNA sequence. Figure 5 showed the differential pulse voltammetric reductive responses of MB at the probe ssDNA modified electrode (curve a) and after hybridization with different kinds of ssDNA sequences. The biggest reduction signal was obtained on the probe ssDNA modified electrode (curve a), which was due to the affinity of MB with the guanine bases on ssDNA molecules that resulted in the greatest amount of MB accumulated on the ssDNA modified electrode. Because the interaction of the probe ssDNA sequence with CTS film was electrostatic attraction due to the opposite charges of two molecules, ssDNA was laid down on the electrode surface randomly. So MB could exhibit a strong affinity to guanine bases in the ssDNA structure and contact most guanines easily, which gave the largest electrochemical response. After hybridization with different ssDNA sequences a dsDNA structure could be formed on the electrode surface. Then the guanine bases were wrapped in the duplex structure of dsDNA and the binding of MB with guanine residue of ssDNA was prevented, so the electrochemical responses decreased gradually [37]. As for the complementary ssDNA sequence the largest decrease of the reduction peak current was observed (curve d), which indicated that the interaction between MB and guanine residues of the probe ssDNA was prevented by the hybridized dsDNA formation on the electrode surface. While the probe ssDNA on the electrode surface was hybridized with three-base mismatched sequence (curve b) and one-base mismatched sequence (curve c), the decrease of the reductive peak current was much smaller than that obtained from the complementary ssDNA sequence. The difference of peak currents (ΔI_p) for three-base and one-base mismatched sequences were 31.9% and 65.9% of that hybridization with the complementary ssDNA sequence, which would be attributed to the partly formed dsDNA structure on the electrode surface. The results were close to the reported values for the three-base mismatched sequence in reference [38] and one-base mis-

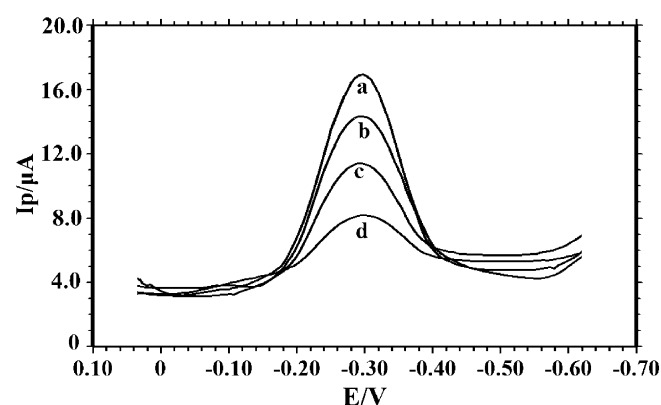


Fig. 5. Differential pulse voltammograms of 20.0 $\mu\text{mol/L}$ MB at the probe ssDNA modified electrode (a) and after hybridization with 1.0×10^{-6} mol/L of three-base mismatched ssDNA sequence (b), one-base mismatched ssDNA sequence (c) and the complementary ssDNA sequence (d).

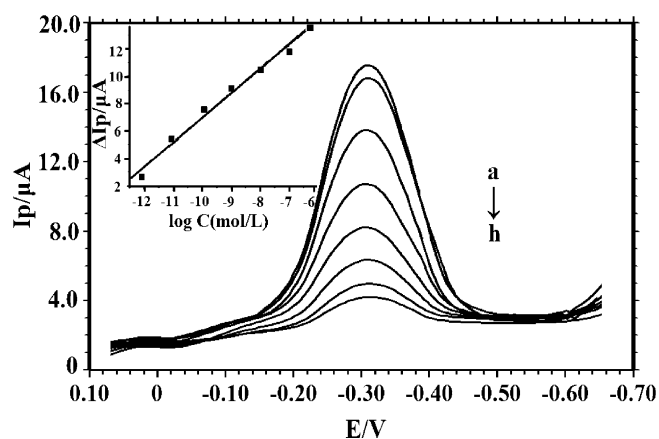


Fig. 6. Differential pulse voltammograms of MB on probe ssDNA modified electrode after hybridization with different concentrations of target ssDNA sequence. Concentrations of target ssDNA sequence from a to h are 0, 1.0×10^{-12} , 1.0×10^{-11} , 1.0×10^{-10} , 1.0×10^{-9} , 1.0×10^{-8} , 1.0×10^{-7} and 1.0×10^{-6} mol/L, respectively. Inset: Plots of ΔI versus logarithm of target ssDNA sequence concentration.

matched sequence in references [39,40]. The results also demonstrated that the fabricated DNA biosensor exhibited high selectivity and good distinguish ability for the different ssDNA sequences with different mismatched bases.

3.6 Sensitivity for Different Concentrations of Target ssDNA Sequence

The sensitivity of this electrochemical DNA biosensor was explored by hybridizing the probe ssDNA modified electrode with different concentrations of target ssDNA sequence related to MON89788 gene of soybean. As displayed in Figure 6, the response of the reduction peak current of MB after hybridization exhibited a gradually decrease with target ssDNA sequence concentrations increasing from 1.0×10^{-12} to 1.0×10^{-6} mol/L and then tended to remain constant, which indicated all the probe ssDNA sequences immobilized on the modified electrode surface had been involved in the hybridization reaction. The decrease of the MB signal was caused by the formed duplex dsDNA structure after the hybrid reaction, which prevented the interaction of guanine bases with MB.

The calibration curve for the detection of the soybean MON89788 ssDNA sequence was constructed in the range from 1.0×10^{-12} mol/L to 1.0×10^{-6} mol/L with the linear regression equation as $\Delta I = 1.79 \log C + 23.2$ ($n=7$, $\gamma=0.992$), where C is the concentration of soybean MON89788 gene sequence (mol/L) and ΔI is the difference of MB peak current before and after hybridization (μA). The detection limit of the MON89788 gene sequence was estimated to be 7.21×10^{-13} mol/L (3σ), which was lower than the previous reports such as nanogold/ZrO₂ film modified GCE (3.1×10^{-11} mol/L) [41], polyaniline modified GCE (1.0×10^{-12} mol/L) [42] and the SH-DNA assembled nanogold modified gold electrode (5.0×10^{-9} mol/L) [43]. The relative standard deviation (*RSD*)

of six independently probe ssDNA modified electrodes fabricated for the measurement of the 1.0×10^{-6} mol/L target ssDNA sequence was 4.3%, indicating the good reproducibility of the modified electrode.

The stability of ssDNA/CTS-GR-TiO₂/CILE was investigated after different storage times at 4 °C and further used to hybridize with the target ssDNA sequence. After 10 days storage of the modified electrode, 96.5% of the initial sensitivity remained. After 20 days storage 93.2% of the initial sensitivity still remained. The results indicated this modified electrode was a stable platform as electrochemical DNA biosensor.

3.7 Detection of PCR Product of Soybean

The hybridization detection for the PCR amplified real sample of soybean samples was further conducted by this electrochemical DNA biosensor under the selected conditions. The PCR amplified samples were diluted with 50.0 mmol/L pH 7.0 PBS, heated in boiling water bath for 10 min to denature and frozen in an ice bath for 2 min immediately. Then 10 μL gene samples were dropped directly onto ssDNA/CTS-GR-TiO₂/CILE for hybridization. After the hybridization reaction the electrode was immersed into MB solution for detection with the results shown in Figure 7. Compared with that of ssDNA/CTS-GR-TiO₂/CILE (curve c), the MB reduction peak current after hybridization with the denatured PCR amplified real sample of MON89788 gene was decreased (curve b), which indicated the good selectivity of this constructed electrochemical DNA biosensor. This significant difference of the MB signals between the probe modified electrode and the hybridized electrode confirmed that this electrochemical DNA biosensor could effectively detect the PCR product of soybean sample.

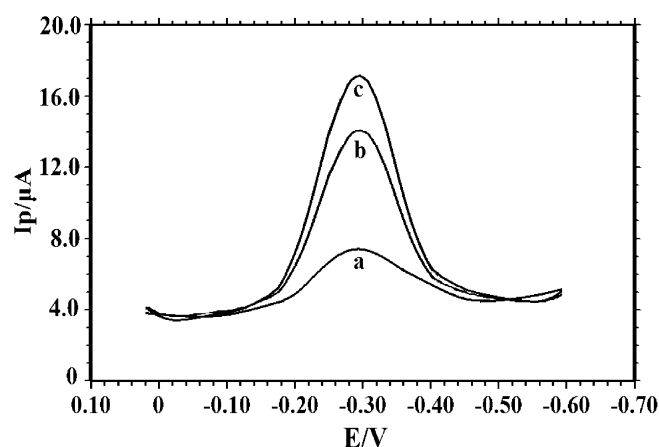


Fig. 7. Differential pulse voltammograms of MB on CTS-GR-TiO₂/CILE (a), ssDNA/CTS-GR-TiO₂/CILE hybridized with PCR product of transgenic soybean MON89788 gene (b) and ssDNA/CTS-GR-TiO₂/CILE (c).

4 Conclusions

In this report we developed a sensitive electrochemical DNA sensor for the detection of the PCR product from transgenic soybean MON89788 gene with a GR and TiO₂ nanorods nanocomposite modified CILE as the substrate electrode. The presence of CTS-GR-TiO₂ nanocomposite on the electrode surface provided a suitable interface for the ssDNA immobilization with increased surface-to-volume ratio, which could increase the amount of ssDNA adsorbed on the electrode. MB could be easily interacted with the guanine bases of ssDNA with a well-defined reduction peak appeared. The fabricated electrochemical DNA sensor was further applied to the sensitive detection of soybean MON89788 target ssDNA sequence and the PCR product from the real soybean samples with a detection limit of 7.21×10^{-13} mol/L (3σ). This electrochemical DNA sensor showed the advantages including simple preparation procedure, good selectivity, wide linear range and high sensitivity. The presence of GR-TiO₂ nanocomposite on the electrode surface provided a large surface area and good electron transfer efficiency with increased loading amount of probe ssDNA sequence, which could greatly improve the detection sensitivity for DNA hybridization. So the investigation extended the application of GR-TiO₂ nanocomposite in the field of electrochemical sensors.

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