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Electrochemical deoxyribonucleic acid biosensor based on carboxyl functionalized graphene oxide and poly-L-lysine modified electrode for the detection of *tlh* gene sequence related to vibrio parahaemolyticus

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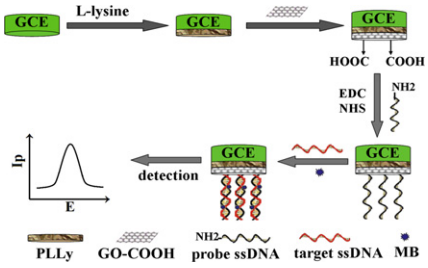
HIGHLIGHTS

- Ploy-L-lysine modified glassy carbon electrode was prepared by electropolymerization.
- A carboxyl functionalized graphene oxide was synthesized and decorated on PLLy/GCE.
- Electrochemical DNA biosensor based on GO-COOH/PLLy/GCE was fabricated.
- The *tlh* gene related to vibrio parahaemolyticus was detected successfully.

GRAPHICAL ABSTRACT



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ABSTRACT

A carboxyl functionalized graphene oxide (GO-COOH) and electropolymerized ploy-L-lysine (PLLy) modified glassy carbon electrode (GCE) was fabricated and used for the construction of an electrochemical deoxyribonucleic acid (DNA) biosensor. The NH_2 modified probe ssDNA sequences were immobilized on the surface of GO-COOH/PLLy/GCE by covalent linking with the formation of amide bonds, which was stable and furthur hybridized with the target ssDNA sequence. Differential pulse voltammetry (DPV) was used to monitor the hybridization events with methylene blue as electrochemical indicator, which gave a sensitive reduction peak at −0.287 V (vs. SCE). Under the optimal conditions the reduction peak current was proportional to the concentration of *tlh* gene sequence in the range from 1.0×10^{-12} to 1.0×10^{-6} mol L^{-1} with a detection limit as 1.69×10^{-13} mol L^{-1} (3σ). The polymerase chain reaction products of *tlh* gene from oyster samples were detected with satisfactory results, indicating the potential application of this electrochemical DNA sensor.

1. Introduction

As a gram-negative bacterium, vibrio parahaemolyticus is commonly found throughout the marine environment, which is the cause of acute gastroenteritis and the source of some cases of septicemia [1]. Human can be infected by this organism through the consumption of raw or undercooked seafood [2]. The *tlh* gene has been observed in all of the vibrio parahaemolyticus strains identified so far, so it is regarded as a useful target for the detection of total vibrio parahaemolyticus [3]. Electrochemical DNA sensors exhibit the advantages such as high sensitivity, selectivity, simplicity, low cost, rapidity of response and ease of miniaturization [4]. Electrochemical detection of DNA hybridization is commonly based on the detection of the current response after the hybridization reaction took place under the selected conditions [5]. So the design and preparation of ssDNA modified electrode

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is of great importance in developing the electrochemical DNA sensor, which can improve the stability, reproducibility and sensitivity [6,7]. Different kinds of transducer surfaces have been devised for the immobilization of probe ssDNA sequence, which can result in the high loading amount of ssDNA concentration on the electrode surface with suitable orientation. In recent year nanoparticles based electrochemical interfaces have been devised for the ssDNA immobilization, which exhibit the advantages such as higher surface area and good biocompatibility with increased conductivity [8]. Hvastkovs and Buttry [9] and Teles and Fonseca [10] reviewed the recent developments in the field of electrochemical DNA sensor.

Poly amino acid film, which exhibits the advantages such as simple and rapid preparation, good stability and reproducibility, has been considered as an excellent alternative to manufacture electrochemical sensors due to the presence of multiple functional groups [11]. Among them ploy-L-lysine (PLLy) has been widely reported as the modifier of the working electrode for electrochemical detection [12,13] and DNA sensor [14,15]. Because amine-containing compounds can be electropolymerized on the surface of carbon electrode [16,17], PLLy can be formed on the electrode surface by electropolymerization, in which L-lysine monomer is oxidized to amino free radical at a higher positive potential and then subsequently forms a carbon-nitrogen linkage at glassy carbon electrode (GCE) surface.

As a two-dimensional sheet of sp^2 conjugated atomic carbon, graphene (GR) has attracted great attentions due to its specific structure and properties such as large surface area, good optical transparency, high thermal conductivity and mechanical strength. Due to its excellent electrochemical conductivity and electrocatalytic activity, GR based electrochemical sensors and biosensors have been reported and reviewed in recent years [18–20]. As a precursor for the large scale production of GR, graphene oxide (GO) is also a nonstoichiometric two-dimensional carbon nanosheets with many oxygen functional groups decorated on the basal planes (hydroxyl and epoxide groups) and edges (carbonyl and carboxyl) of the carbon sheets [21,22]. GO exhibits specific structure features, extraordinary chemical and electrical properties with low toxicity and good hydrophilicity, which has the potential application in biological system [23]. Due to the presence of aromatic domains and multiple oxygen functional groups, GO has also received much attention in the fields of supercapacitors [24], nanocomposites [25] and biosensors [26,27] in recent years. The presence of oxygen groups on the GO surface makes it more active for the further modification, which can improve the solubility and dispersion ability of the matrix. Among them carboxyl functionalized GO or GR has been reported, which can be covalently bond with organic molecules, biological macromolecules and functional materials that contain active groups through the amidation or the esterification reaction. Park and Jung synthesized carboxylated GO composite with Zn, which exhibited good performance in potential range and current density in supercapacitor [28]. Huang et al. studied the electrochemical behavior of adenine and guanine using carboxyl and amino functionalized GR modified electrode [29]. Song et al. found that carboxyl modified GO had intrinsic peroxidase catalytic activity and applied to the glucose detection [30]. Due to the presence of large quantities of carboxyl groups on the surface of GO nanosheet, the derivative COOH can be coupled with other molecules with amide and ester bonds, which extends the application of GO-COOH in different fields.

In this work L-lysine was electropolymerized on the GCE surface by cyclic voltammetry to fabricate a PLLy modified GCE (PLLy/GCE). Then GO-COOH was cast onto the PLLy/GCE surface to form a GO-COOH/PLLy/GCE. The presence of PLLy film on the electrode surface can provide a polymer interface with large surface area and abundant NH_2 group, which can adsorb carboxyl functionalized

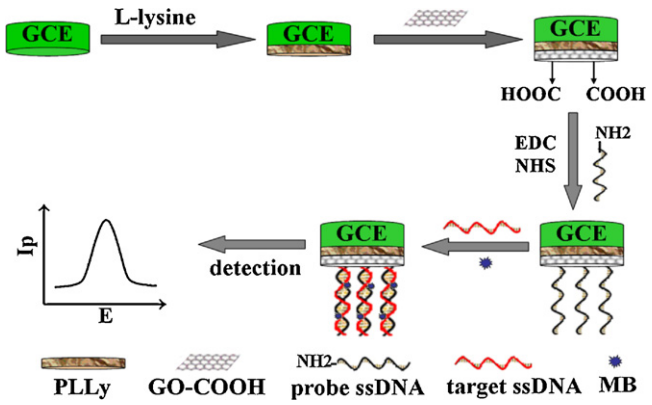

Fig. 1. Fabrication procedure of this electrochemical DNA biosensor.

GO nanosheets that is in negatively charged through electrostatic attraction. Due to the good solubility of GO-COOH, it cannot be stable present on the GCE surface without the presence of PLLy film. So the PLLy film exhibited important roles to establish a polymer interface for the fixation of GO-COOH nanosheets, which was further used as the platform for the immobilization of amine modified probe ssDNA (NH_2-ssDNA) sequence through the formation of amide bonds between carboxyl groups on GO-COOH and amine groups on NH_2-ssDNA. The large surface area of GO nanosheets with many carboxyl groups can increase the amounts of probe ssDNA sequence on the electrode surface, and result in the corresponding increase of the electrochemical responses of MB. The fabrication procedure of this electrochemical DNA biosensor was illustrated in Fig. 1. By using this new electrochemical DNA biosensor, the *tlh* specific ssDNA sequences were successfully detected with a dynamic range from 1.0×10^{-12} to 1.0×10^{-6} mol L^{-1}. The PCR amplification products of *tlh* gene from oyster samples were further detected with the satisfactory result.

2. Experimental

2.1. Apparatus and reagents

A CHI 750B electrochemical workstation (Shanghai CH Instrument, China) was used to carry out all the electrochemical experiments including electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). A conventional three electrode system, which was composed of a saturated calomel electrode (SCE) as reference electrode, a platinum wire as auxiliary and a modified GCE as working electrode, was employed throughout the experiment. Fourier-transform infrared (FT-IR) spectrum was obtained on a Tensor 27 FT-IR spectrophotometer (Bruker, Germany). The DNA extraction kit was purchased from Beijing Tiangen Biotech. Ltd. Co. (China). The PCR reaction was performed on an Eppendorf Mastercycler Gradient PCR system (Eppendorf, Germany).

L-lysine (Shanghai Zhengxiang Chemical Reagent Ltd. Co., China), N-(3-dimethyl-amino-propyl)-N′-ethylcarbodiimide hydrochloride (EDC, Sigma, USA), N-hydroxy-succinimide (NHS, Shanghai Medpep Ltd. Co., China) and methylene blue (MB, Shanghai Chemicals Plant, China) were used as received. Different kinds of buffers used were as follows: 0.2 mol L^{-1} PBS (pH 8.0), 50.0 mmol L^{-1} PBS (pH 7.0), 50.0 mmol L^{-1} Tris–HCl buffer solution (pH 7.0), 1 × TAE buffer (40.0 mmol L^{-1} Tris + 1.0 mmol L^{-1} EDTA + 40.0 mmol L^{-1} acetate, pH 8.0). All the solutions were prepared with doubly distilled water and other chemicals used were of analytical reagents grade.

23 base sequences, which were selected from *tlh* gene sequence, were purchased from Shanghai Sangon Biological Engineering Tech. Ltd. Co. (China) with the sequences as follows:

probe ssDNA: 5′-NH_2-GATGACACTGCCAGATGCGACGA-3′;
target ssDNA: 5′-TCGTCGCATCTGGCAGTGTCATC-3′;
one-base mismatched ssDNA: 5′-TCGTCGCATCTAGCAGTGTCATC-3′;
three-base mismatched ssDNA: 5′-TAGTCGCATCTAGCAGTGTC-AGC-3′;
non-complementary ssDNA: 5′-ATCCTTTGCAATTGCCCAGTCGG-3′

The oligonucleotide primers for PCR amplification reaction of *tlh* gene that extracted from oyster samples were with the following sequences:

Primer F: 5′-AAAGCGGATTATGCAGAAGCACTG-3′;
Primer R: 5′-CGATCTCTTCTTGTGTTGAGTACTTAAACTG-3′

2.2. Synthesis of carboxyl functionalized graphene oxide

Graphene oxide (GO) was synthesized based on the modified Hummer's method [31,32]. Carboxylation of GO was prepared according to the previous report [28,33]. In brief 1.2 g NaOH was added into 5.0 mL of 2.0 mg mL^{-1} GO solution, and the mixture was sonicated for 2 h. Then 1.0 g of chloroacetic acid was added and sonicated for 2 h to convert the hydroxy to carboxyl via conjugation of acetic acid moieties to give carboxyl functionalized graphene oxide (GO-COOH) [34]. Then the resulted GO-COOH solution was repeatedly washed with water and centrifuged until the pH was neutral.

2.3. Fabrication of modified electrode

Prior to modification, GCE was polished on 0.05 μm Al_2O_3 slurries carefully. Then it was rinsed and ultrasonicated in doubly distilled water for 5 min in order to remove any adsorbed substances on the electrode surface. After dried in air at room temperature electropolymerization of L-lysine on GCE was performed based on the reported procedure [35,36]. In general GCE was placed in a 0.2 mol L^{-1} pH 8.0 PBS that contained 0.01 mol L^{-1} L-lysine and cycle voltammetric scans were performed for 10 cycles in the potential range from −1.0 to 2.2 V at a scan rate of 100 mV s^{-1}. Then the electrode was thoroughly rinsed with double distilled water and dried in air at room temperature to get a PLLy modified GCE (PLLy/GCE). Finally a 5.0 μL of 1.0 mg mL^{-1} GO-COOH solution was dropped onto the surface of PLLy/GCE and then dried in air to get the modified electrode, which was denoted as GO-COOH/PLLy/GCE.

2.4. Immobilization of probe ssDNA on GO-COOH/PLLy/GCE

The GO-COOH/PLLy/GCE was immersed into a mixture solution of 5.0 mmol L^{-1} EDC and 8.0 mmol L^{-1} NHS for 30 min to activate the carboxyl group on the electrode interface. Then 5.0 μL of 1.0×10^{-6} mol L^{-1} probe ssDNA (in 50.0 mmol L^{-1} pH 7.0 PBS) were dropped onto the electrode surface, which could be further immobilized onto GO-COOH/PLLy/GCE surface by the formation of covalent amide bond between the carboxyl group on GO-COOH surface and the amine group of ssDNA sequence. Finally, the electrode was washed with 0.5% SDS solution and double distilled water to remove unbound probe ssDNA sequence, and the modified electrode was denoted as ssDNA/GO-COOH/PLLy/GCE.

2.5. Hybridization of target ssDNA sequence

The hybridization reaction was performed by dropping 5.0 μL of different concentrations target ssDNA sequence in 50.0 mmol L^{-1} PBS for 4 h at room temperature. Then the electrode was rinsed with 0.5% SDS solution and double distilled water to remove the non-hybridized target ssDNA sequence. This hybridized electrode was denoted as dsDNA/GO-COOH/PLLy/GCE.

2.6. Electrochemical detection

The hybridized electrode was placed in a 2.0×10^{-5} mol L^{-1} MB solution for 8 min to accumulate MB molecules on the hybrid interface. After washed by 0.5% SDS solution and water for several times to remove the physically adsorbed MB molecules, the electrode was immersed in a 50.0 mmol L^{-1} Tris–HCl buffer solution (pH 7.0) and the electrochemical response of MB was measured by differential pulse voltammetric method with the experimental parameters set as: pulse amplitude 0.008 V, pulse width 0.05 s and pulse period 0.2 s.

2.7. Polymerase chain reaction procedure

The DNA template for PCR amplification was extracted from the oyster samples, which were minced into tiny pieces, added into 10 mL concentrated digestion solution and incubated for 48 h in water bath at 25 °C. Then the solution was centrifuged 4 min at 12,000 rpm to obtain the precipitate, which was dispersed in 100 μL sterilized distilled water immediately. Then DNA sequence was extracted according to the procedure of DNA extraction kit.

The PCR amplification of *tlh* gene was performed according to the following steps. Firstly, 200.0 nmol L^{-1} primer F, 200.0 nmol L^{-1} primer R, 10× reaction buffer B (Promega, Wisconsin, USA), 2.0 mmol L^{-1} $MgCl_2$, 200.0 nmol L^{-1} each of dATP, dCTP, dGTP and dTTP, 1.5 units of Taq DNA polymerase (Promega, Wisconsin USA) and 1.0 μL DNA template extracted from the oyster samples were added in a 0.2 mL reaction tube with the final volume as 25 μL. Secondly, amplification conditions were set 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. Finally, 6 μL of the PCR products were analyzed by electrophoresis separation (5 V cm^{-1}, 40 min) on a 2% agarose gel containing 0.5 μg mL^{-1} of ethidium bromide in 1× TAE buffer. The obtained PCR products of *tlh* gene were kept at 4 °C before use.

3. Results and discussion

3.1. FT-IR spectrum of GO-COOH

FT-IR spectra of the synthesized GO and GO-COOH were recorded and shown in Fig. 2. It can be seen that the specific peaks of hydroxyl (3430 cm^{-1}), carbonyl (1722 cm^{-1}) and epoxy (1330, 1060 cm^{-1}) groups appeared on the spectrum of GO (curve a), which indicated that different kind of oxygen functional groups existed on the GO surface. The result was in consistent with that of the existed groups of GO nanosheet synthesized with the chemical oxidation [22]. While the spectrum of GO-COOH exhibited different absorption shape and strength (curve b) with the enhanced characteristic peak located at 1722 cm^{-1} (C=O stretch vibrations of carboxyl), a strong and broad peak at 3418 cm^{-1} (O—H stretch vibrations of carboxyl). The result indicated that the presence of large amount of carboxyl groups on the GO surface, which was transformed from hydroxyl groups by the reaction with chloroacetic acid.

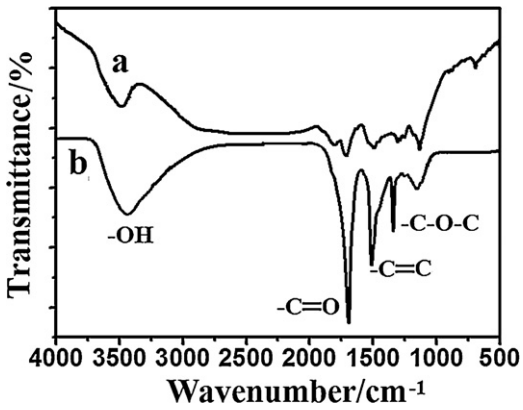

Fig. 2. FT-IR spectra of GO (a) and GO-COOH (b).

3.2. EIS characterization of different modified electrodes

Electrochemical impedance spectroscopy (EIS) has been widely used as an effective method to measure the impedance change of the electrode surface during the modified procedure. The semicircle portion observed at high frequencies corresponds to the electron transfer limited process and its diameter is equal to the electron transfer resistance (R_{et}), which reflects the electron transfer kinetics of the redox probe at the electrode interface [37]. The EIS results of different modified electrodes were recorded and shown in Fig. 3. On bare GCE the Ret value was got as 997.5 Ω (curve a). When L-lysine was electropolymerized onto GCE surface to get a PLLy film, the R_{et} value decreased to 297.1 Ω (curve b), which may be ascribed to the attraction between the positive charge of amino group in PLLy and negative charge of $[Fe(CN)_6]^{3-/4-}$, leading to the accumulation of $[Fe(CN)_6]^{3-/4-}$ on the electrode interface [7]. After GO-COOH was further modified on PLLy/GCE surface, the smallest Ret value of 139.2 Ω was got (curve c), which may be ascribed to the presence of GO-COOH on the electrode surface. GO-COOH exhibited the specific characteristics such as large surface area and good conductivity, which resulted in more $[Fe(CN)_6]^{3-/4-}$ participating in the reaction. Although the electric conductivity of GO-COOH was not as good as that of GR, it still could form a conductive interface, which could increase the electron transfer of $[Fe(CN)_6]^{3-/4-}$ with the Ret value decreased. When the probe ssDNA sequence was immobilized on GO-COOH/PLLy/GCE, the Ret value increased to 437.5 Ω (curve d), which indicated that ssDNA sequence had been fixed on the electrode surface. The negative charges of ssDNA sequences on the electrode surface could hinder the diffusion of negatively charged $[Fe(CN)_6]^{3-/4-}$ probe to the electrode with the Ret value increased.

3.3. Electrochemical behaviors of MB on different modified electrodes

MB is a phenothiazine dye that is commonly used as electrochemical indicator in DNA biosensor [38]. Fig. 4 showed the differential pulse voltammograms of MB on different modified electrodes with GO or GO-COOH as the modifier, which could further prove the function of GO-COOH in the electrode fabrication. The reduction peak of MB appeared at −0.287 V on all the modified electrodes, which was attributed to the electrochemical reaction of phenothiazine group. The reduction peak current of MB on dsDNA/GO-COOH/PLLy/GCE (curve a) was larger than that of dsDNA/GO/PLLy/GCE (curve b), indicating that more dsDNA molecules existed on the electrode surface which could accumulate more MB molecules into the dsDNA structure. Also the reduction peak current of MB on ssDNA/GO-COOH/PLLy/GCE (curve c) was larger than that of ssDNA/GO/PLLy/GCE (curve d). The result could be attributed to the abundant carboxyl group on GO surface, which could supply more activated sites for the immobilization of NH_2-ssDNA on the electrode surface through the formation of amide bond with the help of EDC and NHS. Then more MB molecules could be accumulated on the electrode surface with higher reduction peak appeared. Electrochemical responses of MB were increased greatly on dsDNA/GO-COOH/PLLy/GCE (curve a) than that of ssDNA/GO-COOH/PLLy/GCE (curve c), and the difference of reductive peak currents of MB also indicated that MB was an effective electrochemical indicator to distinguish ssDNA and dsDNA on the electrode surface. In general there are at least three different interaction models between MB and DNA. MB can interact with anionic phosphate framework of DNA by electrostatic binding, the electrostatic and/or intercalation with the major or minor grooves of dsDNA helix, and preferential binding between MB and guanine bases. So the reported results about the electrochemical behaviors of MB on the DNA modified electrode seems contradictory due to the complicated interactions models, which is often related to the DNA sequences or the experimental conditions [39]. For example Ozsoz et al. indicated that MB exhibited large electrochemical signals on the ssDNA modified electrode than the dsDNA modified electrode, which could be attributed to the laid down of DNA sequence on the electrode surface that was benefit for the interaction of MB with guanine bases [40]. While Gao et al. reported an increase of the electrochemical response of MB on the dsDNA modified electrode after the hybridization [41]. Because the probe ssDNA

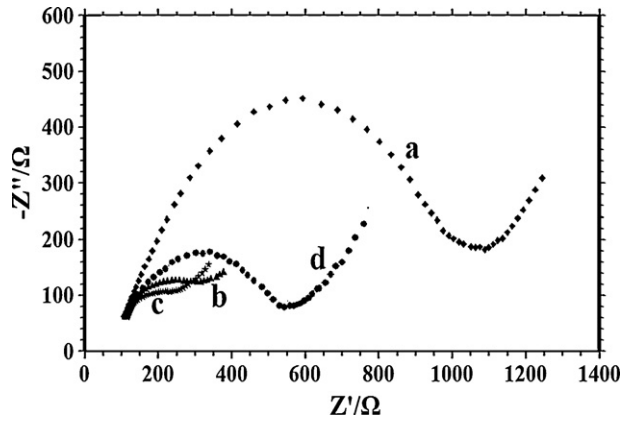

Fig. 3. EIS of different modified electrodes in 1.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol L^{-1} KCl with the frequency sweep from 10^4 to 1.0 Hz (a: GCE, b: PLLy/GCE, c: GO-COOH/PLLy/GCE and d: ssDNA/GO-COOH/PLLy/GCE).

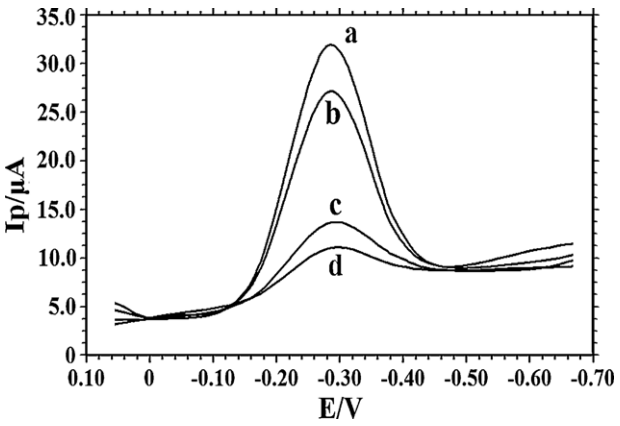

Fig. 4. Differential pulse voltammograms of (a) dsDNA/GO-COOH/PLLy/GCE, (b) dsDNA/GO/PLLy/GCE, (c) ssDNA/GO-COOH/PLLy/GCE and (d) ssDNA/GO/PLLy/GCE using MB as the indicator in 50.0 mmol L^{-1} pH 7.0 Tris–HCl buffer solution.

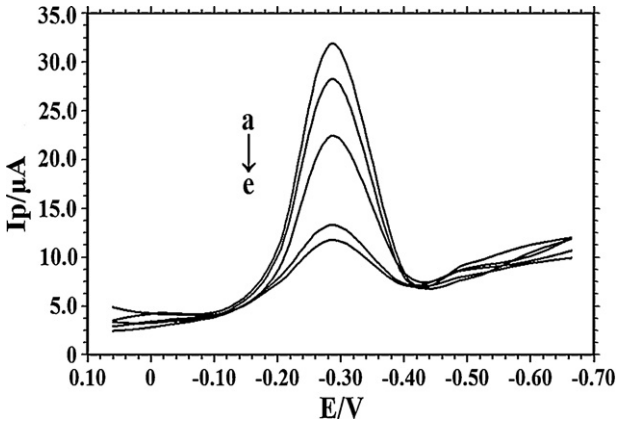

Fig. 5. Differential pulse voltammograms of MB at ssDNA/GO-COOH/PLLy/GCE (e) and after hybridized with 1.0×10^{-6} mol L^{-1} complementary ssDNA sequence (a), one-base mismatched ssDNA sequence (b), three-base mismatched ssDNA sequence (c) and noncomplementary ssDNA sequence (d).

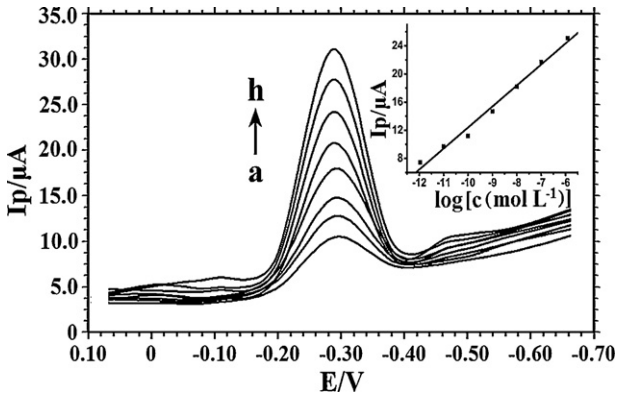

Fig. 6. Differential pulse votlammograms of MB on ssDNA/GO-COOH/PLLy/GCE after hybridization with different concentrations of target ssDNA sequence (from a to h were 0, 1.0×10^{-12}, 1.0×10^{-11}, 1.0×10^{-10}, 1.0×10^{-9}, 1.0×10^{-8}, 1.0×10^{-7} and 1.0×10^{-6} mol L^{-1}, respectively). Inset: plots of I versus logarithm of target ssDNA concentration.

sequence was assembled on GO-COOH/PLLy/GCE surface through covalent bonds, which was present in a vertical approach on the electrode. After hybridization MB could intercalate into the dsDNA double helix, which gave the bigger electrochemical responses than that of ssDNA modified electrode.

3.4. Selectivity of the electrochemical DNA biosensor

The selectivity of this electrochemical DNA biosensor was explored by using the ssDNA/GO-COOH/PLLy/GCE to hybridize with different ssDNA sequences with electrochemical responses of MB recorded and shown in Fig. 5. It can be seen that the biggest reduction signal was observed when ssDNA/GO-COOH/PLLy/GCE was hybridized with complementary ssDNA sequence (curve a), indicating that the formation of hybridized dsDNA on the electrode surface had a strong interaction with MB. After hybridization with one-base mismatched ssDNA sequence (curve b) or three-base mismatched ssDNA sequence (curve c), the reductive peak current was much smaller than that obtained from the complementary ssDNA sequence, indicating the partly formed dsDNA led to the smaller amount of the intercalated MB molecules. At the same time the peak current of the hybridization with the one-base mismatched ssDNA sequence gave a higher value than that of three-base mismatched ssDNA sequence, indicating the good distinguish ability for the hybridization reaction. After hybridization with the noncomplementary sequence (curve d) the electrochemical response of MB gave the smallest increase than that obtained on ssDNA/GO-COOH/PLLy/GCE, indicating no hybridization reaction took place and the little increase of the peak current may be attributed to the non-specific adsorption of ssDNA on the electrode surface. These results exhibited that this electrochemical DNA sensor performed good selectivity to different ssDNA sequences.

3.5. Sensitivity of the electrochemical DNA biosensor

The sensitivity of this electrochemical DNA sensor was investigated by using the probe ssDNA modified electrode to hybridize with different concentrations of the complementary ssDNA sequence. The more the hybridization of probe ssDNA sequence with target ssDNA sequence, the larger amount of dsDNA formed on the electrode surface, the more the MB molecules intercalated and the bigger electrochemical response can be observed. As shown in Fig. 6, the reduction current response of MB increased with the concentration of complementary target ssDNA sequence from 1.0×10^{-12} to 1.0×10^{-6} mol L^{-1}. The relationship of reductive peak currents with the logarithmic value of the target ssDNA sequence could be plotted with the linear regression equation as $Ip(\mu A) = 2.985 \log[c/(\text{mol L}^{-1})] + 42.24$ ($n = 7$, $\gamma = 0.994$). The detection limit was calculated as 1.69×10^{-13} mol L^{-1} (3σ) (where σ is the RSD of the blank solution, $n = 11$), which was lower than the previous reported values such as the PLLy/SWCNT modified carbon paste electrode (3.1×10^{-13} mol L^{-1}) [7], chitosan/Fe_3O_4 microsphere-GR composite modified CILE (3.59×10^{-13} mol L^{-1}) [42] and multiwalled carbon nanotubes and gold nanoparticles modified gold electrode (7.5×10^{-12} mol L^{-1}) [43]. The detection limit was larger than some recent reported values of 3.4×10^{-14} mol L^{-1} [44] and 3.5×10^{-14} mol L^{-1} [45] with the usage of gold nanoparticle decorated GR sheets modified electrode. The reason may be due to the high conductive interface with the Au–GR nanocomposite, which was better than GO-COOH used in this paper. The relative standard deviation (RSD) of the reductive peak current for the 6 repeated detections of 1.0×10^{-7} mol L^{-1} target ssDNA sequence was calculated as 3.9%, indicating the good reproducibility of this method. The stability of ssDNA/GO-COOH/PLLy/GCE was investigated after 10 days storage at 4 °C and further used to hybridize with the target ssDNA sequence, 96.8% of the initial sensitivity remained, indicating this modified electrode was a stable platform as electrochemical DNA biosensor.

3.6. Detection of PCR products of tlh gene sequence

The PCR products from oyster sample were denatured by heating the solution in a boiling water bath for 10 min and then frozen in an ice bath for 2 min to obtain the ssDNA solution, which were further analyzed by the proposed method. By dropping 5.0 μL solution on the surface of ssDNA/GO-COOH/PLLy/GCE with the following hybridization and electrochemical detection performed under the same procedures, differential pulse voltammograms were recorded and shown in Fig. 7. The reduction peak current of MB increased greatly after hybridization with the denatured PCR sample of *tlh* gene (curve c). While the hybridization with the PCR amplified sample without DNA template gave little increase (curve b). The results indicated that large copies of DNA sequence were formed under the PCR amplification, which could give large concentrations of target ssDNA sequences after denaturation. After hybridization more dsDNA molecules were formed on the electrode surface, which could accumulate more MB on the electrode surface with biggest response. The little increase of MB peak current on the curve b than that of curve a may be caused by the non-specific adsorption effect of ssDNA sequence in the PCR solution. This significant difference of the reductive peak current of MB between the

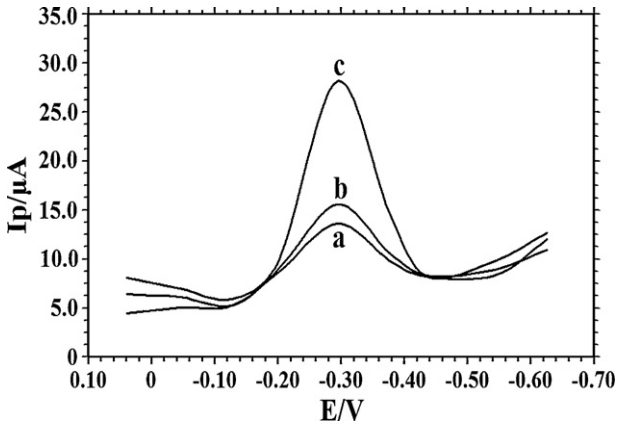

Fig. 7. Differential pulse voltammograms of MB at ssDNA/GO-COOH/PLLy/GCE (a) and hybridized with the PCR product without (b) and with (c) DNA template of *tlh* gene sample.

probe ssDNA modified electrode (curve a) and hybridized dsDNA electrode (curve c) also confirmed that this electrochemical DNA biosensor could be effectively applied to detect the PCR product of *tlh* gene.

4. Conclusions

GO-COOH was successfully synthesized and combined with electropolymerized poly-L-lysine (PLLy) modified GCE to give a platform for the further immobilization of probe ssDNA sequence. The fabricated electrochemical DNA sensor exhibited better performance due to the presence of GO-COOH and PLLy, which provided a carboxyl functionalized interface with high surface area and good conductivity. The *tlh* gene sequence related to vibrio parahaemolyticus was successfully detected by this electrochemical DNA sensor in the concentration range from 1.0×10^{-12} to 1.0×10^{-6} mol L^{-1} with a detection limit as 1.69×10^{-13} mol L^{-1} (3σ). The electrochemical DNA sensor showed excellent discrimination ability to the detection of different ssDNA sequences and was further applied to the PCR product of *tlh* gene extracted from oyster sample.

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