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Electrochemical detection of a Vibrio parahaemolyticus sequence-specific gene based on a gold electrode modified with a single stranded probe

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An electrochemical DNA biosensor for specific-sequences detection of Vibrio parahaemolyticus (VP) was fabricated. A single-stranded 20-mer oligonucleotide (ssDNA) and 6-mercapto-1-hexanol (MCH) were immobilized via a thiol linker on gold disk electrodes by self-assembling. The ssDNA underwent hybridization in a hybridization solution containing complementary or non-complementary or single base pair mismatched DNA sequences of VP. Examination of changes in response to these three target DNAs showed that the developed biosensor had a high selectivity and sensitivity.

Vibrio parahaemolyticus, electrochemical DNA sensor, self-assembling, gold electrode

1 Introduction

Vibrio parahaemolyticus (VP), a gram negative bacterium distributed worldwide in the estuarine environment [1–3], causes acute gastroenteritis in humans when consumed in raw, undercooked or mishandled seafood [4].

Although conventional methods such as polymerase chain reaction (PCR) amplification [5, 6], enzyme-linked immunosorbant assay (ELISA) [7] are possibilities for VP detection, these methods require specialized equipment, employing time-consuming processes, making them less suitable for rapid detection. Electrochemical DNA sensors [8–10] significantly shorten the time without extensive pre-treatment steps, frequently utilized for the detection of bacteria [11, 12]. However, studies of electrochemical DNA sensors for detection of VP have rarely been reported. It is unclear that the use of electrochemical DNA sensors for VP detection is effective.

Different kinds of solid electrodes, e.g. metal and carbon electrodes, have been employed as transducers for electrochemical DNA sensors [13]. Among these electrodes, gold electrodes have specific advantages, including a wide potential window at the cathode region and a smooth surface. The strong affinity of the Au-S bond makes use of thiol based self assembled mono-layers (SAMs) [14, 15] for the immobilization of a single-stranded DNA probe on a gold electrode [16–18, 11]. The electrode surface is polishable and renewable.

The efficient prevention of space steric hindrance to insure high hybridization efficiency is an issue in the fabrication of reliable electrochemical DNA sensors. 6-mercapto-1-hexanol (MCH) has been shown to be a suitable molecule employed as an interstitial spacer, which is used to reduce nonspecific absorption of the DNA probes and also to control the probe density on the substrate [19, 20]. Because

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MCH is an uncharged and flexible molecule with a highly inert interface, it is unlikely to interact significantly with DNA.

In our work, we aimed to consider a method based on an electrochemical DNA sensor by immobilizing thiol modified probes/MCH membrane for distinguishing specific sequences of VP. The hybridization events were transformed into electrochemical signals [21–24] that manifest the presence of specific DNA target sequences.

2 Experimental

2.1 Apparatus and electrodes

Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) measurements were performed with a CHI 440A Electrochemical Analyzer (CHI Instruments Inc., USA). The three-electrode system consisted of a gold disk working electrode (CHI Instruments Inc., 2.0 mm diameter), an Hg/Hg_2Cl_2 reference electrode (saturated KCl) and a counter electrode made of platinum wire. All electrochemical measurements were conducted at room temperature and 0.05 M PBS (pH 7.2) was used as the electrolyte in all experiments.

UV-vis absorption spectroscopy was performed with a UV-2102 (Unico (Shanghai) Instrument Co., Ltd., USA).

2.2 Reagents and solutions

Hoechst 33258 and 6-mercapto-1-hexanol (MCH) were purchased form Sigma-Aldrich Chemical Co. (USA). H33258 stock solution was prepared with ultra-pure water and kept in the dark at 0–4 °C and used up within seven days. Phosphate buffer solution (PBS, pH 7.2) and TE (10 mmol/L Tris-HCl, 1 mmol EDTA pH 8.0) were used. Other reagents were all of analytical reagent grade without any additional purification. All of the solutions were prepared with ultra-pure water.

Twenty-base synthetic oligonucleotides were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China): 20-mer probe sequence (ssDNA): 5′-thiol-(C6)-GAT GTC AGC CAT TTA GTA CC-3′; 20-mer complementary DNA sequence: 5′-GGT ACT AAA TGG CTG ACA TC-3′; 20-mer one-base mismatch sequence: 5′-GGT ACT AAA TCG CTG ACA TC-3′; 20-mer non-complementary sequence: 5′-TCC TAA GCT CTA TGA CAT AT-3′.

DNA oligonucleotide stock solutions (100 μM) were prepared in a TE buffer solution and kept frozen at –20 °C. A more dilute solution of the probe was prepared in a 0.05 M PBS containing 0.1 M NaCl (pH 7.2), and other oligomers were diluted in 0.05 M PBS containing 0.3 M NaCl (pH 7.2).

The efficient immobilization of thiolated DNA probes depends on their purity. Thus the probe solution is detected by UV-vis absorption spectrometry before any experiment begins. The ratio between the absorbance values obtained at 260 and 280 nm which were close to 1.8 indicated that DNA was sufficiently free of protein, and that the probe was suitable for electrochemical deposition at the electrode surface [11].

2.3 Procedures

2.3.1 Pretreatment of the gold electrode

Gold disk electrodes were polished with 0.05 μm alumina, before being respectively ultrasonicated in ultra-pure water and ethanol each for 5 min. The Au electrode was further treated electrochemically in a 0.5 M H_2SO_4 solution by scanning the potential from –0.2 to 1.7 V at a scan rate of 100 mV/s until an ideal voltammogram for a clean Au was observed.

2.3.2 Thiolated probe immobilization

The electrodes were exposed to a 1 μM thiol-modified probe ssDNA solution for 2 h at room temperature. After DNA immobilization, the gold electrodes were treated with a 1.0 mM 6-mercapto-1-hexanol solution for 1 h at room temperature to reduce non-specific DNA binding. The modified electrodes were rinsed with PBS for 5 s and then thoroughly rinsed with ultra-pure water. The immobilization processes of ssDNA self-assembling onto a gold electrode and the DNA detection method are shown in Diagram 1.

2.3.3 Probe hybridization

A hybridization reaction was carried out by immersing the modified electrode into a hybridization solution containing 0.025 μM target DNAs for 1 h at 29 ± 2 °C. It was subsequently rinsed three times respectively with phosphate buffer and ultra-pure water.

2.4 Indicator binding and electrochemical measurement

The ssDNA or dsDNA modified electrode was immersed in

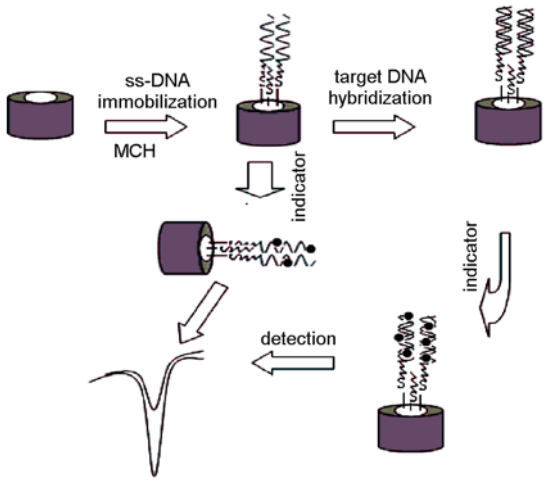

Diagram 1 The schematic illustration of a DNA biosensor.

a Hoechst 33258 aqueous solution (50 μM) for 10 min to deposit the indicator on the electrode. The electrode was washed with ultra-pure water to remove the physically adsorbed molecules. The oxidation signals of the ssDNA and dsDNA were measured by LSV and their peak currents were regarded as the analytical signal.

In addition, typical cyclic voltammetry (CV) experiments were performed in a 2 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ aqueous solution at room temperature.

3 Results and discussion

3.1 Characterization of the bare and probe/MCH-modified electrode

We chose six gold electrodes in this study. In order to avoid correcting the difference of the anodic current signal of the indicator in subsequent experiments, it was necessary to ensure that the active surface areas of all the electrodes were identical [25]. Following pretreatment of the electrodes, CV experiments were performed in a 2 mM ferricyanide aqueous solution containing 0.5 M KCl. The peak currents of the six curves were nearly indistinguishable (with RSD of 2.8%), indicating that the effective surface areas of all the electrodes was nearly the same (data not shown).

For the sake of showing the immobilization of the DNA probe onto the gold surface, we also used the ferricyanide system (Figure 1). A bare gold electrode had typical redox peaks (curve a) in a 2 mM ferricyanide aqueous solution containing 0.5 M KCl. However, when the DNA probe was immobilized onto the gold surface by self-assembling and utilizing MCH as a spacer, the peak currents of ferrocyanide/ferricyanide decreased and the peak-to-peak separation increased (curve b), due to the fact that the surface became so negatively charged (due to phosphate groups along the DNA backbone) that it retarded the anionic ferricyanide from approaching the electrode surface. Therefore, the DNA probe immobilization on the gold surface was demonstrated. In addition, with Hoechst 33258, the peak current of the ssDNA modified gold electrode was higher than the bare electrode (Figure 2), which also indicated that the DNA probe was immobilized on the gold electrode [26].

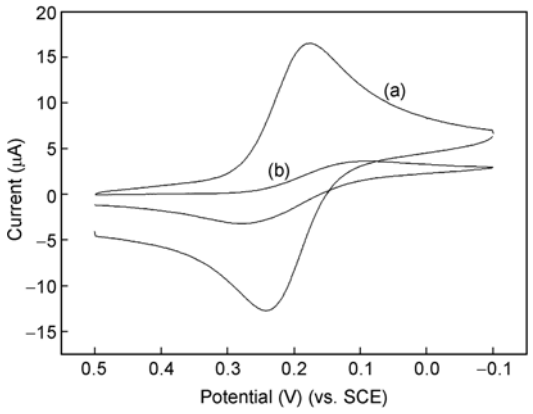

Figure 1 Cyclic voltammograms measured with a bare gold electrode (a) and an ssDNA-MCH mixed monolayer (b) modified gold electrode in the 2 mM ferricyanide aqueous solution containing 0.5 M KCl, with a scan rate of 50 mV/s.

3.2 Comparison between pure DNA-SH and DNA-SH/MCH mixed monolayers

Preparation of mixed monolayers of a thiol-modified DNA probe and a spacer (MCH), by the way of a two-step method, was described by Herne and Tarlov [19]. After MCH post-treatment, thiol-linked oligonucleotides were vertical on the surface. Two different fabrications of probe-modified electrodes were simultaneously processed, one had MCH as spacer, and the other omitted MCH. Both fabrications of sensors were then exposed to a hybridization solution containing 1 μM complementary DNA for 1 h at 29 ± 2 °C. Figure 3 shows the corresponding current signal. When the MCH treatment was omitted and hybridization was directly attempted, the oxidation signal of the ssDNA was higher (5.03 μA) (Figure 3(A), curve a) because of more non-specific adsorption and the probe lying on the electrode surface, thus resulting in much lower hybridization efficiency with target DNA. The significant improvement in sensitivity that followed MCH treatment was attributed to sharp changes in the probe interfacial bio-architecture, with the oxidation signal of ssDNA being 1.81 μA (Figure 3(B), curve a), with corresponding improvement in hybridization efficiency.

3.3 Influence of sodium chloride concentration on the self-assembling solution

The density of the probe at the gold surface is closely related to the salt concentration of the self-assembling solution, time and temperature. 0.05 M PBS containing 0 M, 0.1 M, 0.3 M sodium chloride were prepared for the SH-ssDNA

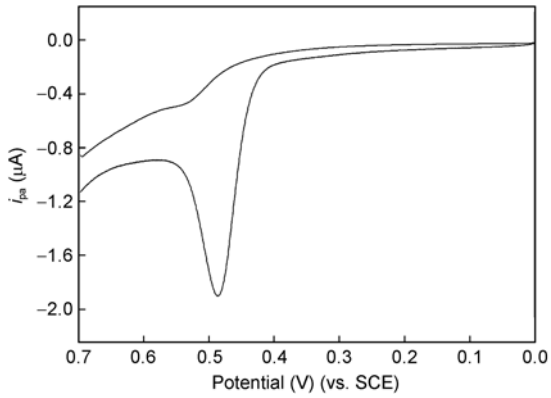

Figure 2 Cyclic voltammograms measured with a bare gold electrode (a) and an ssDNA-MCH mixed monolayer (b) modified gold electrode in the 2 mM ferricyanide aqueous solution, with a scan rate of 50 mV/s.

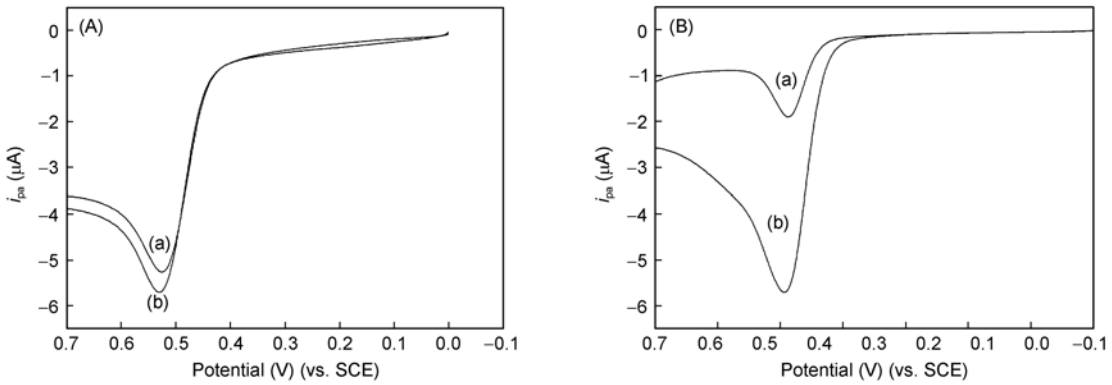

Figure 3 Linear sweep voltammograms of modified gold electrodes. Curves in panel A: (a) SH-ssDNA modified electrode; (b) hybridized with its complementary sequences. Curves in panel B: (a) SH-ssDNA/MCH modified electrode; (b) hybridized with its complementary sequences.

self-assembling, with results shown in Figure 4. The surface probe density was increased with increasing salt concentration, but the hybridization efficiency increased to the maximum at 0.1 M. The current difference before and after hybridization with complementary DNA was about 3.47 μA when the salt concentration was 0.1 M, which was wider compared with other conditions, indicating that the salt concentration of the immobilization solution had a significant impact on hybridization efficiency.

3.4 Specificity of DNA detection

The probe hybridized with three target DNAs (complementary DNA, non-complementary DNA and single base pair mismatched DNA) with a concentration of 0.025 μM. Figure 5 presents the LSVs of the electrode modified with ssDNA (before hybridization) and each dsDNA (after hybridization) in 0.05 M PBS (pH 7.2).

There was a large difference in the anodic peak currents (i_{pa}) between the ssDNA and hybridization with the complementary target DNA. The i_{pa} value of the ssDNA modified electrode was 1.86 μA (RSD = 2.9%, $n = 3$), which increased to 4.11 μA after hybridization (RSD = 5.5%, $n = 3$). A limited change in the LSV response was exposed to the non-complementary oligonucleotide. The RSD value for the peak current obtained in this case was 5.2% ($n = 3$). Concerning the single-base mismatch sequence (curve c), the anodic current signal was lower than the target DNA, with the RSD value being 4.2% ($n = 3$). This indicated a much lower hybridization efficiency of the single-base mismatch and the noncomplementary DNA compared with the complementary target DNA, thus showing the sensor's ability to detect a Vibrio parahaemolyticus sequence-specific gene.

As shown in Figure 6, an almost linear correlation between the target sequence concentration and the electrochemical signal was found within the 0.025 to 1 μM range. The regression equation was $I_p/\mu A = 1.2101\ C_{ssDNA}/\mu M + 4.0439$ (I_p was the LSV peak current; C_{ssDNA} was the concentration of the target complementary ssDNA), and the regression correlation coefficient (R^2) of the calibration line was 0.9959. The detection limit of the biosensor was 6.25 pmol (250 μL of 0.025 μM).

4 Conclusions

This paper developed an electrochemical DNA sensor for

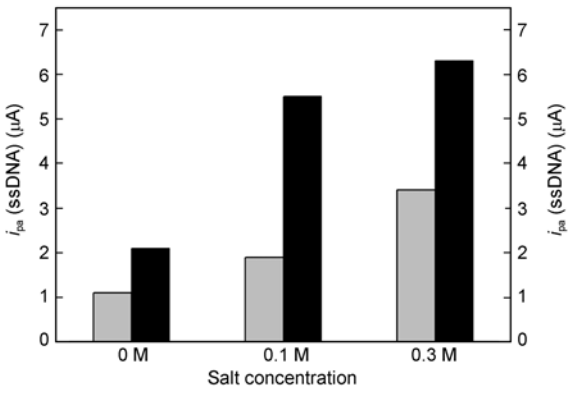

Figure 4 Current change before and after hybridization with electrodes immersed in different salt concentrations of the SH-ssDNA solution (target DNA concentration: 1 μM).

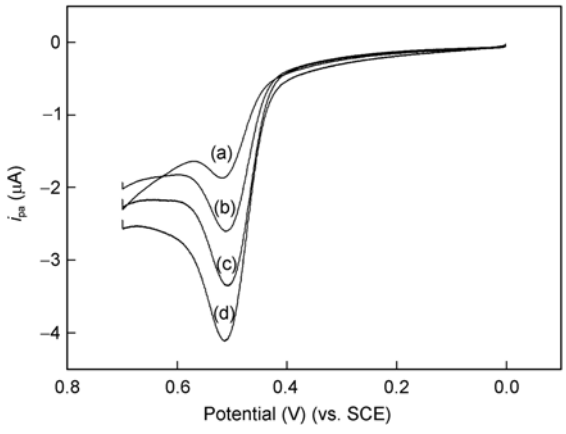

Figure 5 Linear sweep voltammograms of (a) ssDNA/Au and after hybridization with (b) noncomplementary, (c) single base mismatch sequence, (d) complementary oligonucleotide sequence. Sweep rate: 100 mV/s.

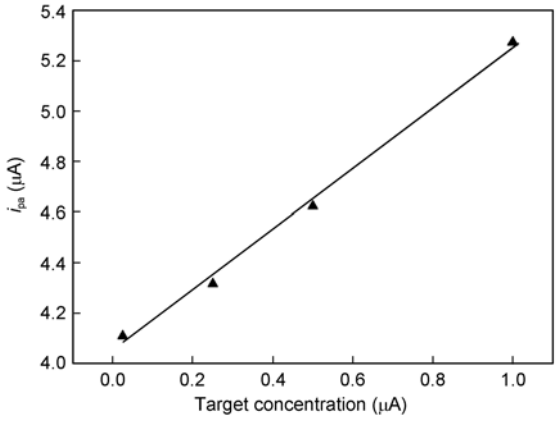

Figure 6 Linear relationship: probe-modified electrodes were exposed to target DNA solutions 0.025, 0.25, 0.5, 1 μM in a hybridization buffer.

rapid detection of specific-sequences of VP based on DNA hybridization on mixed monolayers of a thioled probe and an MCH modified gold electrode. The entire fabrication process of the sensor was simple, but high sensitivity and selectivity were achieved by LSV experiments with H33258 as the indicator. The results showed that this method qualitatively and quantitatively detected specific sequences of VP. Although the electrochemical DNA sensor was applied to VP as a model, it will be extended toward the detection of other pathogenic bacteria in food.

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