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An Electrochemical Sensor Based on Allosteric Molecular Beacons for DNA Detection of *Escherichia Coli.* O157:H7

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In this work, an E-sensor basing on allosteric molecular beacons (aMBs) was designed for detection of *Escherichia coli.*（*E. coli.*）O157:H7 DNA. Without the target DNA, the aMB formed a stable hairpin structure which blocked the binding capability of the streptavidin (SA) aptamer. After incubating with target DNA, the hairpin opened and the SA aptamer was reactivated to capture SA-HRP. The HRP enzyme catalyzed the TMB by H_2O_2 to form TMB^{2+}, which was reduced at the E-sensor surface to generate an electrochemical current signals concern the target DNA. It was found that the reaction was more pronounced in pH 5.0 buffer. The E-sensor had the best response in 20 mmol/L H_2O_2 and 0.8 mmol/L TMB, and had the best response in 6 minutes. The E-sensors showed a good linear of *E. coli.* O157:H7 DNA from 1×10^{-9}g/ml to 1×10^{-6}g/ml. It Show a good linear relationship between the logarithms of *E. coli.* O157:H7 DNA and current peak height of DPV (y=2.3501ln(x)-0.8836, x: DNA, Y: peak height of DPV). It show a good regression coefficient (R^2=0.9885) too. Furthermore, it can realize the quantitative detection of *E. coli.* O157:H7 DNA.

Keywords: Electrochemical sensor; Molecular beacons; DNA detection; *E. coli.* O157:H7

1. INTRODUCTION

DNA detection has been observed in many types of infectious diseases and cancers. It has become increasingly important in a wide range of areas including infectious bacteria diagnosis, disease prevention and environmental monitoring [1]. In recent years, the foodborne diseases have emerged as major concerns of public and increasingly threaten the health of people. Among the pathogenic microorganisms, *E. coli.* O157:H7 is one of the most harmful food pathogens which can cause serious foodborne illnesses and deaths [2]. It is reported that the infectious dose of *E. coli.* O157:H7 is possibly less than 100 colony-forming units (CFU) [3]. *E. coli.* O157:H7 detection at low

concentration is always challenging and requires long time culture before analysis. So, it is of extreme importance to develop new techniques to rapidly and sensitively detect *E. coli.* O157:H7 from food samples.

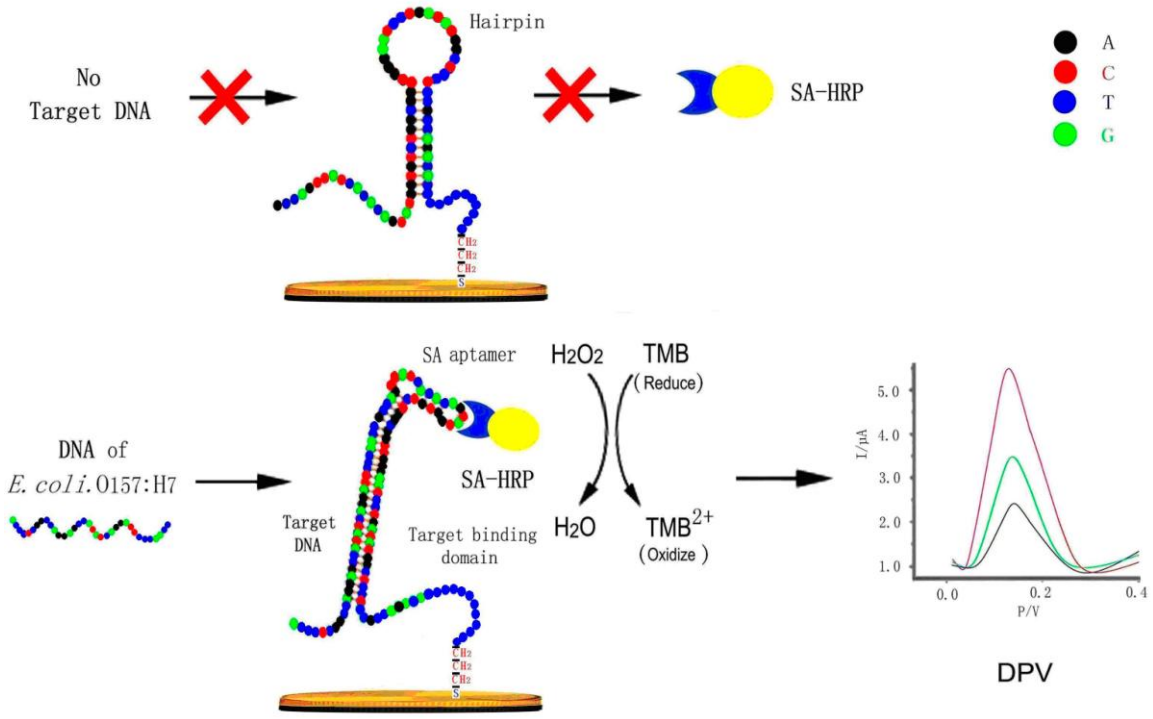
Among various detection methods for nucleic acids, the electrochemical sensor (E-sensor) has unique and attractive strengths including high sensitivity, low cost, simplicity and portability. Solid state biosensors have been developed for rapid detection of bacteria as alternatives to the traditional methods such as enzyme-linked immunosorbent assay (ELISA) and culture and colony counting method [4]. Various solid state biosensing techniques have been used for *E. coli.* O157:H7 detection such as quartz crystal microbalance (QCM) [5] and surface plasmon resonance (SPR) [6] and electrochemical biosensor [7]. In an effort to identify natural antimicrobial agents as fresh produce-wash, the effect of essential oils in reducing enteric pathogens on iceberg and romaine lettuce was investigated [8]. A novel surface plasmon resonance (SPR) biosensor using lectin as bioreceptor was developed for the rapid detection of *E. coli.* O157:H7 [9]. Ensafi *et al.* [10] had developed an electrochemical sensor was for guanine and adenine detection using multiwall carbon nanotubes decorated with $NiFe_2O_4$ magnetic nanoparticles on a glassy carbon electrode. Aptamer is a particular oligonucleotide that can bind to a specific target molecule. It can bind proteins and other molecules with high affinity and specificity, which is comparable with an antibody [11].

In 2009, a solid-state electrochemiluminescence (ECL) biosensing switch system based on special ferrocene-labeled molecular beacon aptamer (Fc-MBA) has been developed successfully for thrombin detections [12]. Biofunctional magnetic beads were investigated for bacterial cells concentration in a nanoporous alumina membrane based immunosensor for ultra-sensitive detection of *E. coli.* O157:H7. This method via magnetic bead conjugation and concentration demonstrated the ultrasensitivity of 10 CFU/mL for *E. coli.*O157:H7 detection [13]. In 2011, Song *et al.* [14] reported an E-sensor based on aMB for DNA detection, which contained a SA aptamer sequence blocked by its hairpin structure. After incubation with a target DNA, the hybridization between the DNA and the aMB caused the stem to open and the formation of SA aptamer structure, which activated the ability of the aMB for binding SA. Wu *et al.* [15] reported a reagentless and reusable "signal-on" electrochemical DNA sensor that utilizes oligo-thymine as a flexible spacer. This sensor design is sensitive, specific, and selective; it is potentially generalizable for detection of a wide range of biomedical-relevant nucleic acid sequences [16]. Farjami *et al.* [17] found that the truncation of the MB down to 20 nucleotides allowed switching of the E-sensor response from the on-off to the off-on mode. They applied this signal-on strategy for cancer diagnostics and investigated the mechanism of electron transfer between the electrode and the DNA-conjugated redox probe. Hybridization of a target DNA to the longer arm of the hairpin displaced the shorter arm with a methylene blue tag, allowing the methylene blue to approach the electrode surface for electron transfer. Meanwhile, to further improve the sensitivity of detection and lower down the limit of detection, enzyme based amplification strategy has been introduced to MB E-sensor system [18].

However, these strategies require sophisticated DNA modification, though they show good selectivity and sensitivity for DNA detection. In addition, the activity of enzymes on the DNA strands is difficult to control, resulting in low reproducibility for detection. An activity-controllable probe is necessary to fabricate a facile and stable E-sensor. Most of the current electrochemical sensors use

metal electrodes as the sensing platforms which suffer from the low sensitivity for bacteria detection due to the electrode polarization effect.

In this study, we developed an E-sensor basing on aMBs for DNA detection of *E. coli.* O157:H7 (Scheme 1). Without the target DNA, the aMB formed a stable hairpin structure which blocked the binding capability of the SA aptamer. After incubating with target DNA, the hairpin opened and the SA aptamer was reactivated to capture SA-HRP. The HRP enzyme catalyzed the oxidation of tetramethylbenzidine (TMB) by H_2O_2 to form TMB^{2+}, which was reduced at the electrode surface to generate electrochemical current signals which reflected quantitative information concerning the target DNA. Based on the experiment，to optimize the reaction conditions and detection methods of the E-sensor, and explore the standard curve of DPV and the linear range of the test.



Scheme 1. The principle of the E-sensor for DNA detection of *E. coli.* O157:H7.

2. EXPERIMENTAL

2.1. Materials

The SA-HRP polymer was purchased from Sigma-Aldrich (Shanghai, China); bovine serum albumin (BSA) from Sangong Inc. (Shanghai, China); TMB from Tiangen (Beijing, China); The DNA absorbance at 260 nm was measured on a Nanodrop ND-2000 (Thermo Scientific, USA) for calculation of the DNA concentration. All designed oligonucleotides were syntheticed by Shanghai synthesized by Sangong Inc (Shanghai, China) and dissolved in buffer (10 mM Tris-HCl, pH 8.0).

2.2. aMB sequence design for E. coli. O157:H7

The aMB sequence of E-sensor consisted of four domains: an SA aptamer domain, a target binding domain, a blocking domain that hybridizes with part of the SA aptamer sequence for temporally blocking its binding towards SA, and an assistant domain that anchors the aMB of gold electrode surface through gold-thiol chemistry and keeps the aMB active sites away from the surface to reduce steric hindrance. The sequences of aMB: 5′–ATTGACCGCTGTGTGACGCAACACTCAAT AACCAAAGGCTTCAGCGCAATCTTCTTATTGAGTGTTTTTTTTTTTT-$(CH_2)_3$-SH–3′. *E. coli.* strain 7 perosamine synthetase (rfbE) gene (Genbank: JQ907523.1) was selected to be target gene. The target DNA of *E. coli.* O157:H7 was desgined as fellow: 5′–GTTTGTAATTGAAGATTGCGCTGAA GCCTTTGGTT–3′. The postive DNA control had been BLAST by National Center for Biotechnology Information (NCBI, USA) and meet the specific requirement of the E-sensor.

2.3. E-sensor modification and cyclic voltammetry test

The SPE gold electrode 1208210 (Metrohm, Spain) was cleaned in 0.5 mol/L H_2SO_4 solution, rinsed with water and dried in a nitrogen flow. The aMB probes at concentration of 0.5 μmol/L were heated to 90 °C for 5 minutes and then treated with 100 μl (1 mmol/L) bovine serum albumin (BSA) (Sigma, USA) for blocking the residual gold active sites. The electrode was extensively washed, and hybridized with the 100 μl （1×10^{-6}g/ml, pH6.0 PBS）DNA of *E. coli.* O157:H7 in 5 minutes, then reacted with 1 ml (40 mmol/L) H_2O_2 and 1 ml TMB (1.0 mmol/L) in15 minutes. At last, the TMB^{2+} was detection by cyclic voltammetry (CV) method. Electrochemical measurements were performed on a CHI 650d electrochemical analyzer (CHI Co., China).

2.4. Optimization of reaction conditions

First, the optimal pH of TMB^{2+} detection was test by cyclic voltammetry (pH from 4.0 to 7.0). Then the electrode was extensively washed and hybridized with the 100μl DNA of *E. coli.* O157:H7 （1×10^{-6}g/ml, pH6.0 PBS）in 5 minutes, reacted with 1 ml different gradient H_2O_2 (from 0 to 80 mmol/L) and 1 ml different gradient TMB (from 0 to 3.2 mmol/L) in different time (from 0 to 8 minutes). At last, the TMB^{2+} was detection by DPV of CHI 650d electrochemical analyzer. The optimal reaction parameters were selected by the results of the experiment.

2.5. Standard curve and linear range of DPV

The electrode was extensively washed and hybridized with the 100μl different gradient DNA of *E. coli.* O157:H7 （from 1×10^{-9}g/ml to 1×10^{-6}g/ml, pH6.0 PBS）in 5 minutes, then reacted with 1.0 ml H_2O_2 and 1.0 ml TMB in same condition (The optimal reaction parameters from up tests were applied in this experiment). At last, the TMB^{2+} was detection by DPV of CHI 650d electrochemical

analyzer. The curves drawing and regressions analysis were executed in OriginPro 7.5 software (OriginLab, USA).

3. RESULTS AND DISCUSSION

3.1. The modification of electrode and preliminary detection

An E-sensor using label-free functional allosteric molecular beacons (aMBs) with enzymatic signal amplification was designed for detection of *E. coli.* O157:H7 DNA. Without the target DNA, the aMB formed a stable hairpin structure which blocked the binding capability of the SA aptamer. After incubating with target DNA, the hairpin opened and the SA aptamer was reactivated to capture SA-HRP. The HRP enzyme catalyzed the oxidation of TMB by H_2O_2 to form TMB^{2+}, which was reduced at the electrode surface to generate electrochemical current signals which reflected quantitative information concerning the target DNA. Cyclic voltammetry (CV) was used to characterize the *E. coli.* O157:H7-aMB E-sensor in reaction solution. The results were shown in Figure 1.

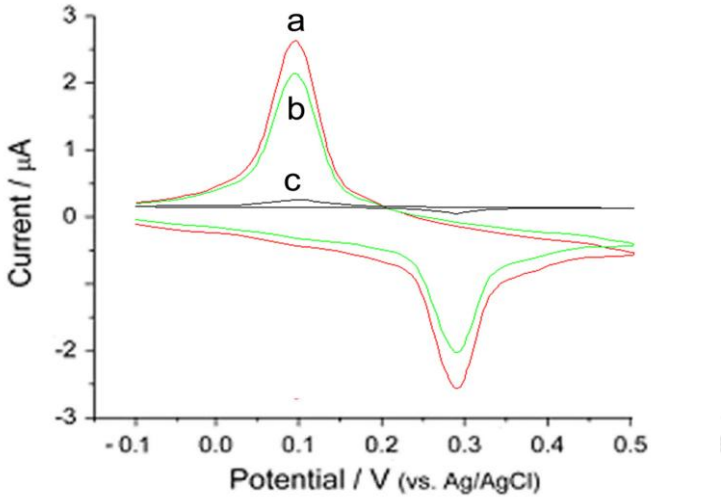


Figure 1. Cyclic voltammetry of E-sensor responding to *E. coli.* O157:H7 DNA. Performed in the reaction solution at100 mV/s. (a: positive control, b: *E. coli.* O157:H7, c: negative control)

The application of electrochemical measurements of the redox couples of metal-based antitumor potential drugs in the presence of DNA is a highly sensitive method due to the resemblance between the electrochemical and biological reactions. Cyclic voltammetry provides a useful complement to the other biophysical methods of investigations such as UV-visible spectroscopy and fluorescence studies. Since the redox active metal complexes are not amenable to such methods either due to weak absorption bands viz; forbidden d-d transitions or because of overlap of electronic transitions with those of DNA molecule, they can potentially be studied via voltammetric techniques [19]. In our previous studies [20], cyclic voltammetry also have similar results.

It suggested that the DNA detection of *E. coli.* O157:H7 could be carried out by the E-sensor basing on aMBs. Therefore, we choose DPV method which had higher resolution to detect the DNA of *E. coli.* O157:H7. The peak potential was selected in 0.1V. Of course, the detection conditions must further optimized through the fellow experiments.

3.2. Optimization of reaction conditions

The results were shown in Figure 2. First, the optimal pH of TMB^{2+} detection was test by cyclic voltammetry from 4.0 to 7.0 (Figure 2-A). It suggested that the reaction was more pronounced in pH 5.0 buffer. When pH was too high, the TMB ionization was not complete, so the clutter peaks were appear in CV. It was the likely cause of detection interference. The different gradient H_2O_2 from 0 to 80 mmol/L were compared in Figure 2-B. It was shown that the E-sensor had the best response in 20 mmol/L H_2O_2. The different gradient TMB from 0 to 3.2 mmol/L were compared in Figure 2-C. It was shown that the E-sensor had the best response in 0.8 mmol/L TMB. The different reaction time from 0 to 8 minutes were compared in Figure 2-D. It was shown that the E-sensor had the best response in 6 minutes.

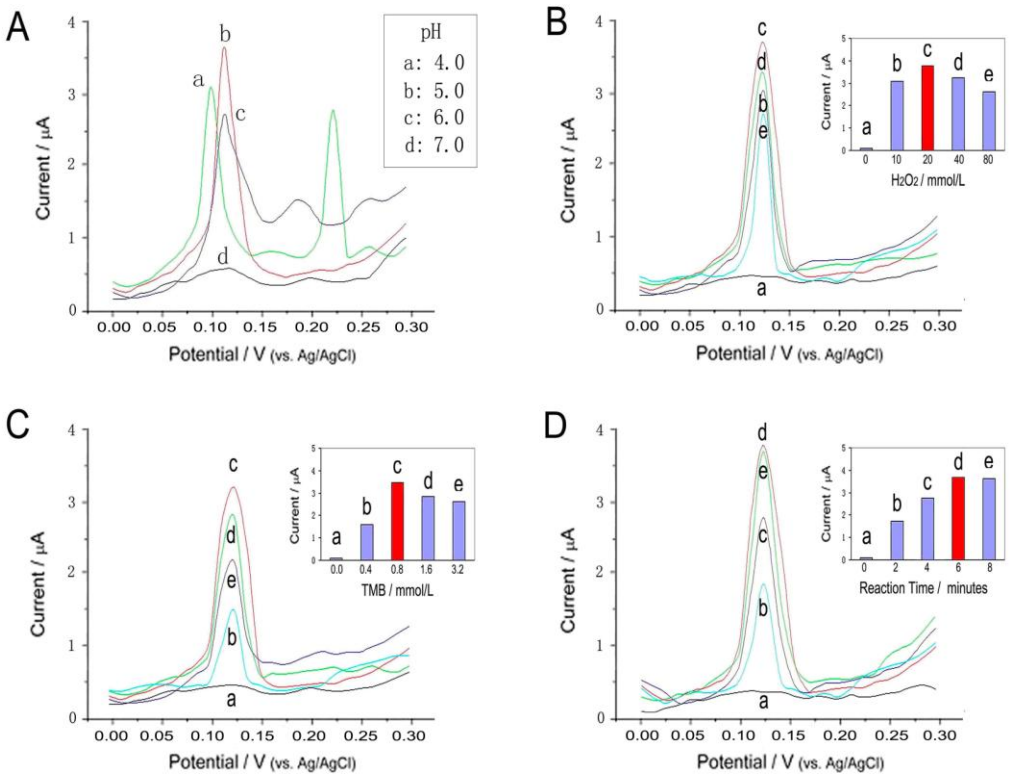


Figure 2. Optimization of reaction conditions to *E. coli.* O157:H7 DNA detection by DPV. (A: pH. a:4.0, b:5.0, c:6.0, d:7.0. B: H_2O_2.a:0, b:10, c:20, d:40, e:80 mmol/L. C:TMB. a:0, b:0.4, c:0.8, d:1.6, e:3.2 mmol/L. D: Reaction time. a:0, b:2, c:4, d:6, e:8 minutes). Target DNA: all were 1×10^{-6}g/ml DNA of *E. coli.* O157:H7.

Research reported that electrochemical sensor has high sensitivity, strong specificity, and was simple and quickly, was more and more applied in DNA detection. Iiu *et al.*[21] reported that highly sensitive and selective colorimetric detection of Ag(I) ion using 3,3 ',5,5 ',-tetramethylbenzidine (TMB) as an indicator. It leading to a simple approach to colorimetric detection of Ag^+ with a detection limit of 50 nM. Linman *et al.* [22] reported a detection of low levels of *E. coli.* in fresh spinach by surface plasmon resonance spectroscopy with a TMB-based enzymatic signal enhancement method.

This study also found similar results. Furthermore, we got the best reaction condition of the E-sensor for DNA detection of *E. coli.* O157:H7. It was applied in the fellow tests.

3.3. Standard curve and linear range of DPV

The E-sensor was hybridized with the 100μl different gradient DNA of *E. coli.* O157:H7 （ from 1×10^{-9}g/ml to 1×10^{-6}g/ml, pH6.0 PBS） in 5 minutes, then reacted with 1.0 ml (20 mmol/L) H_2O_2 and 1.0 ml TMB (0.8mmol/L, pH 5.0) and deleted after reacted 6 minutes. The TMB^{2+} was detection by DPV of CHI 650d electrochemical analyzer. The curves drawing and regressions analysis were executed in OriginPro 7.5 software (OriginLab, USA). The results were shown in Figure 3.

It Show a good linear in Figure 3-A of *E. coli.* O157:H7 DNA from 1×10^{-9}g/ml to 1×10^{-6}g/ml. In Figure 3-B, it shown a good linear relationship between the logarithms of *E. coli.* O157:H7 DNA and current peak height of DPV (y=2.3501*ln(x)-0.8836, x: DNA, Y: peak height of DPV). It Show a good regression coefficient (R^2=0.9885) of *E. coli.* O157:H7 DNA too.

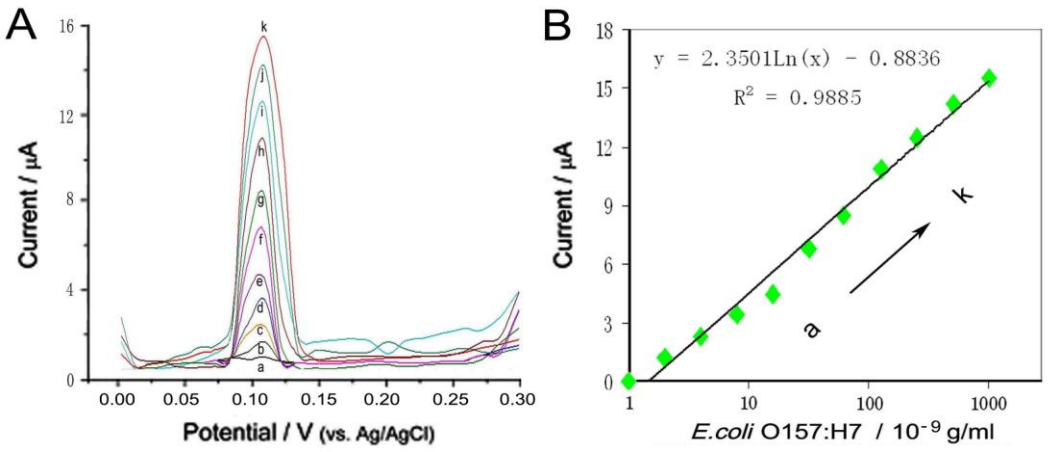


Figure 3. Standard curve and linear range of *E. coli.* O157:H7 DNA. A: DPVs of E-sensor detection of different gradient *E. coli.* O157:H7 DNA. B: Regressions analysis of current peak heights of DPVs vs logarithms of *E. coli.* O157:H7 DNA (a:1, b:2, c:4, d:8, e:16, f:32, g:64, h:128, i:256, j:512, k:1024×10^{-9}g/ml)

Bi *et al.* [23] introduce a simple but robust label-free method to detect DNA based on large-scale gold nanoplate (GNP) films with tunable localized surface plasmon resonance (LSPR) and highly surface-enhanced Raman scattering (SERS) activity. It shows the highest signal intensity with SERS

enhancement factor (EF) as high as 5.4×10^7 and also has excellent stability, reproducibility and repeatability. The optimized SERS-active substrate with the largest enhancement ability could be used to detect double-strand DNA without a dye label, and the detection limit is down to 10^{-9} g/ml.

Ghanim *et al.* [24] had Design a disposable DNA biosensor microchip with amperometric detection featuring PCB substrate. Electrochemical detection (ECD) is one of the best means for designing a Lab-on-a-chip system especially for electroactive analyte's separation and detection applications like biomedical and in vitro diagnostic. The new design will help producing low cost Lab-on-a-chip devices for DNA or other electroactive diagnostic purposes. With sensitivity consistently higher than 100 nA and separation time of approximately 30 minutes, this new design is an ideal tool for real DNA analysis.

Our study also found similar results. The sensitivity of the E-sensor was high and the linear range was wide. Furthermore, it can realize the quantitative detection of *E. coli.* O157:H7 DNA.

4. CONCLUSIONS

In this work, an E-sensor using label-free functional aMBs was designed for detection of *E. coli.* O157:H7 DNA. Without the target DNA, the aMB formed a stable hairpin structure which blocked the binding capability of the SA aptamer. After incubating with target DNA, the hairpin opened and the SA aptamer was reactivated to capture SA-HRP. The HRP enzyme catalyzed the oxidation of TMB by H_2O_2 to form TMB^{2+}, which was reduced at the electrode surface to generate electrochemical current signals which reflected quantitative information concerning the target DNA.

The results suggested that the DNA detection of *E. coli.* O157:H7 could be carried out by the E-sensor basing on aMBs. Then the detection conditions must further optimized through the fellow experiments. We found that the reaction was more pronounced in pH 5.0 buffer. When pH was too high, the TMB ionization was not complete, so the clutter peaks were appear in CV tests. It was the likely cause of detection interference. The different gradient H_2O_2, TMB and reaction time were compared. It was shown that the E-sensor had the best response in 20 mmol/L H_2O_2 and 0.8 mmol/L TMB. And the E-sensor had the best response in 6 minutes after reacted.

The E-sensors showed very good sensitivity and selectivity to the targets. It had shown a good linear of *E. coli.* O157:H7 DNA from 1×10^{-9}g/ml to 1×10^{-6}g/ml. It has shown a good linear relationship between the logarithms of *E. coli.* O157:H7 DNA and current peak heights of DPV ($y=2.3501*\ln(x)-0.8836$, x: DNA, Y: peak height of DPV). It had shown a good regression coefficient ($R^2=0.9885$) of *E. coli.* O157:H7 DNA too. This type of aMB E-sensor eliminated the antigen–antibody interaction which requires sophisticated DNA modification. It can realize the quantitative detection of *E. coli.* O157:H7 DNA.

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