J. Iran. Chem. Soc., Vol. 7, No. 1, March 2010, pp. 119-127.

JOURNAL OF THE
Iranian
Chemical Society

Electrochemical DNA Biosensor for the Detection of *Listeria Monocytogenes* Using Toluidine Blue as a Hybridization Indicator

H.-W. Gao[a,*], P. Qin[b], C. Lin[a], Z.-M. Shang[b] and W. Sun[b]

[a]*Shandong Entry-Exit Inspection and Quarantine Bureau of People's Republic of China, Qingdao 266002, P.R. China*

[b]*College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P.R. China*

(Received 9 November 2008, Accepted 30 January 2009)

An electrochemical DNA biosensor was established for the determination of actin-assembly inducing protein (actA) gene sequences from *Listeria monocytogenes* and its polymerase chain reaction (PCR) product. The actA gene probe sequences were covalently immobilized on the surface of the mercaptoacetic acid self-assembled gold electrode with the help of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS), which was further used to hybridize with the target sequence. Toluidine blue (TB) was used as an effective electrochemical indicator for the discrimination of the hybridization reaction on the electrode surface, which had stronger interaction with double-stranded DNA (dsDNA) than single-stranded DNA (ssDNA). The electrochemical parameters of TB on DNA modified electrodes were carefully calculated. Based on the different electrochemical responses of TB on DNA modified electrodes, the actA gene sequences can be detected in the concentration range from 1.0×10^{-7} to 8.0×10^{-5} M. The PCR product of *Listeria monocytogenes* was successfully detected by the proposed electrochemical biosensor.

Keywords: Actin-assembly inducing protein gene, Toluidine blue, Polymerase chain reaction, Electrochemical DNA biosensor, Hybridization indicator

INTRODUCTION

Electrochemical DNA biosensor has aroused great interest in recent years for its simplicity, higher sensitivity and cheaper equipments [1-3], and has enormous potential applications for the specific sequences detection in human disease, clinical test, biochemical analysis and so on. Electrochemical transducers are powerful tools for the detection of DNA hybridization reaction. Most of them are based on the immobilization of an oligonucleotide sequence on the electrode surface and the detection on the differences of an electroactive indicator with ssDNA or dsDNA modified electrodes [4-6].

Recently some reviews have focused on the development of electrochemical DNA biosensor and its application. For example Lucarelli *et al*. discussed the carbon or gold electrode as electrochemical transducers for DNA hybridization sensors [7]. Wang *et al*. applied nanoparticles as a new label for the electrochemical DNA detection [8]. Generally speaking, the design of electrochemical DNA biosensor often involves the following steps [9,10]: (1) the immobilization of DNA probe sequence on the electrode surface; (2) the hybridization with the target sequence; (3) the electrochemical detection with or without indicator. Among them, the first step is most important because it will influence the overall performances of the

*Corresponding author. E-mail: ghw75@126.com

electrochemical sensor. The higher reactivity, orientation and stability of the surface-controlled probe sequence will facilitate the maximization of the hybridization efficiency and the minimization of the non-specific adsorption.

Many methods have been proposed for the probe immobilization such as chemical absorption, covalent binding, avidin-biotin system, molecular self-assembly and nanoparticles [11-15]. The hybridization is often exhibited *via* the redox signal of an electrochemical indicator, which can be an organic molecules, metal complexes, enzymes, redox labels or nanoparticles [16-19].

Listeria monocytogenes is considered as one of the most hazardous, potentially life-threatening, human foodborne pathogens. It can contaminate many food products, such as milk, cheese, ice cream, raw vegetables, poultry products, and meats [20]. The development of rapid, sensitive, simple and cost effective methods to detect this pathogen is extremely important in implementing an effective response to the prevention of foodborne diseases.

Toluidine blue (TB) is a cathonic phenothiazine dye with well-defined electrochemical redox behaviors, which can interact with DNA in the solution. Passmore *et al*. used spectroscopic method for the DNA detection by using TB as the probe [21]. Sun *et al*. studied the interaction of TB with herring fish dsDNA and established a voltammetric method for the determination of dsDNA in synthetic samples [22]. The electrochemical behaviors of TB on the ssDNA and dsDNA modified electrodes were investigated in this paper and the results showed that the electrochemical response of TB on ssDNA and dsDNA modified electrodes could be discriminated, which was used as a new electrochemical indicator. The proposed method was applied to the detection of specific actin-assembly inducing protein (actA) gene sequences from *Listeria monocytogenes* and further combined with polymerase chain reaction (PCR) method for the the PCR product detection.

EXPERIMENTAL

Apparatus

All the cyclic voltammetric measurements were performed on an LK 98A electrochemical workstation (Tianjin Lanlike Chemical and Electron High Technology Co. Ltd., China) with a three-electrode system consisting of a bare gold or DNA modified gold working electrode, a saturated calomel (SCE) reference electrode and a platinum wire auxiliary electrode. The PCR amplification was performed on an Eppendorf Mastercycler Gradient PCR system (Eppendorf, Germany). A pHS-25 acidimeter (Shanghai Leici Instrument Factory, China) was used to control the pH of buffer solution.

Reagents

Toluidine blue (TB, Shanghai Reagent Company), mercaptoacetic acid (MAA, Tianjin Yuanhang Reagent Company), N-hydroxysuccinimide (NHS, Shanghai Reagent Company), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, Sigma) and native fish sperm double-stranded DNA (dsDNA, Beijing Jingke Reagent Company) were used as received. The denatured fish sperm single-stranded DNA (ssDNA) was produced by heating the native dsDNA solution in a 100 ºC water bath for 10 min and then rapidly cooled in an ice-water bath. All the other reagents were of analytical reagents grade and doubly distilled water was used throughout.

The following buffers were prepared for the experiments: phosphate buffer solution (PBS, 20.0 mM KH_2PO_4 + NaOH, pH 7.0), saline sodium citrate (2×SSC) hybridization buffer (300.0 mM NaCl + 30.0 mM sodium citrate tribasic dihydrate, pH 7.0). Tris-EDTA buffer solution (TE, 10.0 mM Tris-HCl + 1.0 mM EDTA); 0.2 M Britton-Robinson (B-R) buffer solution (pH 6.0).

Preparation of Electrochemical DNA Biosensors

The gold electrode surface was pretreated step-by-step before the modification process. Firstly, the gold electrode was heated in a Piranha solution (30% H_2O_2 and concentrated H_2SO_4 in 3:7) for 5 min and then polished with 0.05 μm albumin/water slurry to get a mirror-like surface. Then the electrode was sonificated in ethanol and water for 2 min, respectively. The freshly pretreated gold electrode was dipped into a 10.0 mM mercaptoacetic acid (MAA) solution for 24 h to get the MAA self-assembled gold electrode. After that, the electrode was rinsed three times with water to remove the physically adsorbed MAA, and the resulting modified electrode was denoted as MAA/Au electrode.

The MAA/Au electrode was immersed in pH 7.0 PBS

containing 5.0 mM EDC and 8.0 mM NHS for 30 min to get a Linker/MAA/Au electrode, then dipped in ssDNA solution (pH 7.0 PBS at 25 ºC) for 12 h. At this step the covalent bonding between the carboxyl groups and 3'-hydroxy end of ssDNA sequence occurred to form a carboxylate ester and ssDNA was tightly immobilized on the surface of gold electrode, which was denoted as ssDNA/MAA/Au.

The ssDNA/MAA/Au electrode was further hybridized with target ssDNA sequence in 2×SSC buffer solution for 1 h at 60 ºC and then cooled to 25 ºC. After hybridization, the electrode was rinsed by water and a dsDNA/MAA/Au electrode resulted, which was stored in a pH 8.0 TE buffer solution at 4 ºC before use.

Electrochemical Measurements

By immersing the DNA modified electrode in 1.5×10^{-4} M TB at pH 6.0 B-R buffer solutions for 5 min and scanning in the potential range from 0.1 V to -0.4 V by cyclic voltammetry with the scan rate as 100 mV s^{-1}, the electrochemical responses of the accumulated TB on the DNA modified electrode surface were recorded. All the analytical results were the average value of three parallel measurements.

Polymerase Chain Reaction Procedure

Total 6 *Listeria monocytogenes* and 9 other bacterial strains used in this study are listed in Table 1. All experiments reported here involved bacteria grown overnight in Luria broth, inoculated into Luria broth, and grown to logarithmic phase as measured by OD 600. DNA from the other aliquot of the bacterial dilution was extracted using a DNA extraction kit (Beijing Tiangen Biotech. Company, China).

The PCR primers sequences used in the current study were designed by the Primer 5.0 software program according to the sequences present in the actA gene (GenBank access number EF542823.1, EF542822.1, EF542820.1, EF542821.1). The primers used in PCR reaction included: L-actA Primer 1: 5'-CGGCTCTGATAAGTGACAT-3'; L-actA Primer 2: 5'-GCTTGTTTGTTTCTGCTTGTT-3'. The Primer 1 of L-actA was also used as the actA probe sequence immobilized on the gold electrode with the above procedure. Then the

Table 1. List of Bacterial Strains and their Sources Used in the *Listeria Monocytogenes* PCR Experiment

Listeria monocytogenes	IQCC22201	Donated by CAIQ
Listeria monocytogenes	IQCC22202	Donated by CAIQ
Listeria monocytogenes	IQCC22203	Donated by CAIQ
Listeria monocytogenes	IQCC22204	Donated by CAIQ
Listeria monocytogenes	IQCC22205	Donated by CAIQ
Listeria monocytogenes	IQCC22206	Donated by CAIQ
Listeria monocytogenes	ATCC15313	Purchase from ATCC
Listeria ivanovii	ATCC19119	Purchase from ATCC
Listeria grayi	ATCC25401	Purchase from ATCC
Listeria seeligeri	ATCC35967	Purchase from ATCC
Listeria innocua	ATCC33090	Purchase from ATCC
Listeria welshimeri	ATCC35897	Purchase from ATCC
Staphylococcus aureus	IQCC22045	Donated by CAIQ
Yersinia enterorcolitica	IQCC10901	Donated by CAIQ
Salmonella aberdeen	IQCC12310	Donated by CAIQ
Shigella flexneri	IQCC10130	Donated by CAIQ

[a]CAIQ, Chinese Academy of Inspection and Quarantine, Beijing, China. [b]ATCC, American Type Culture Collection 10801 University Boulevard Manassas (VA), USA.

complementary target, noncomplementary ssDNA and the two-based mismatched ssDNA had the following base sequence: 5'-ATGTCACTTATCAGAGCCG-3', 5'-CGATTGCAG CATTACGCAC-3' and 5'-ATGTCACATATCAGAGGCG-3', respectively. All the oligonucleotide sequences were synthesized by Shanghai Shanjing Biotech. Company.

Amplification of actA DNA fragments was carried out in an 0.2 ml tube with the final tube volume of 25 µl containing 200.0 nM each primer of L-actA Primer 1 and L-actA Primer 2, 10×reaction buffer B (Promega, Wisconsin USA), 2.0 mM $MgCl_2$, 200.0 nM each of dATP, dCTP, dGTP and dTTP; 1.5 units of Taq DNA polymerase (Promega, Wisconsin USA), 1.0 µl DNA template purified from samples.

During the PCR procedure, DNA was initially denatured at 94 ºC for 30 s. PCR conditions were optimized as follows: 35 cycles of amplification (94 ºC for 30 s, 56 ºC for 30 s, 72 ºC for 30 s) and final extension at 72 ºC for 5 min 6 µl of each of the PCR products was 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 (40.0 mM Tris, 1.0 mM EDTA, 40.0 mM acetate, pH 8.0), and visualized with UV transilluminator.

RESULTS AND DISCUSSION

Cyclic Voltammograms of TB on Different Modified Electrodes

The interaction of TB and dsDNA in the solution was studied by cyclic voltammetry, linear sweep voltammetry and UV-Vis adsorption spectrophotometry. The results indicated that the interaction mode of TB with DNA was caused by electrostatic binding under the selected condition [23]. The electrochemical data of the interaction of TB with dsDNA and ssDNA in solution are listed in Table 2. TB had a pair of symmetrical redox peaks on the bare gold electrode (curve 1 of Fig. 1). After the addition of 100.0 mg l^{-1} ssDNA into TB solution, the anodic and cathodic peak current of TB decreased by 49.3% and 16.3% with little negative movement of the peak potential, whereas the addition of 100.0 mg l^{-1} dsDNA into TB solution resulted in great decrease of peak current by 85.8% and 46.8% with the negative shift of the peak potential, which was the typical electrostatic binding mode of DNA interaction. The results indicated that both ssDNA and dsDNA showed electrostatic binding interaction with TB. The higher decrease of the peak current upon the addition of dsDNA compared with that of ssDNA indicated that dsDNA had higher affinity with TB than ssDNA.

Figure 1 shows the cyclic voltammograms of 1.5×10^{-4} M TB on different modified electrodes in pH 6.0 Britton-Robinson (B-R) buffer solution at the scan rate of 100 mV s^{-1}. Curve 1 is that of TB on the bare gold electrode with a pair of symmetrical redox peaks appearing, which is due to the electrochemical reaction of TB on the gold electrode. Curve 2 is that of TB on MAA/Au with the redox peak currents decreased apparently and the peak potential negatively shifted. The peak-to-peak separation (ΔE_p) was calculated as 42 mV, which was bigger than that of TB, indicating that it was difficult for TB to take place with redox reaction on the surface of MAA/Au electrode.

The formation of MAA self-assembled monolayer hindered the electrochemical reaction of TB and then resulted in the decrease of peak current. Curve 3 is that of TB on ssDNA/MAA/Au electrode with the redox peak currents increased, which indicates that ssDNA immobilized on the surface of MAA/Au electrode interacted with TB in the solution. The redox peak potentials were negatively moved with the E_{pa} as -0.181 V and the E_{pc} as -0.219 V, which is the characteristic of the electrostatic attraction [24]. Curve 4 is that of TB on dsDNA/MAA/Au electrode with the redox peak

Table 2. Electrochemical Data of 1.5×10^{-4} M TB with ssDNA and dsDNA in Solution

Solution	E_{pa} (mV)	E_{pc} (mV)	I_{pa} (nA)	I_{pc} (nA)	Decrease in I_{pa}	Decrease in I_{pc}	ΔE_p (mV)
TB	-0.184	-0.216	-4.665	5.790	-	-	32
TB+ssDNA	-0.182	-0.236	-2.365	4.845	49.3%	16.3%	54
TB+dsDNA	-0.190	-0.252	-0.664	3.078	85.8%	46.8%	62

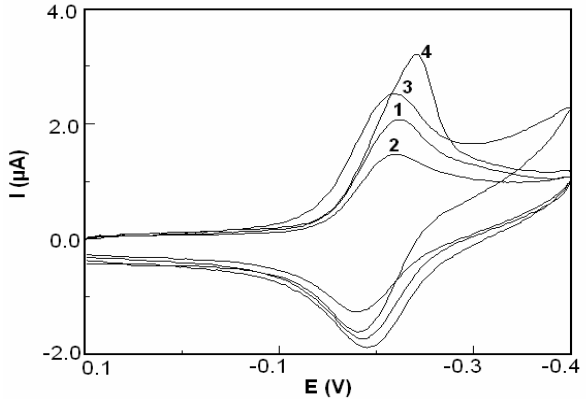

Fig. 1. Cyclic voltammograms of 1.5×10^{-4} M TB on bare Au (1), MAA/Au (2), ssDNA/MAA/Au (3) and dsDNA/MAA/Au (4) with scan rate as 100 mV s^{-1}.

current increased greatly.

The redox peak potentials negatively moved to -0.191 V (E_{pa}) and -0.245 V (E_{pc}), respectively, indicating the electrostatic binding of TB with dsDNA [23]. The results indicated that TB showed stronger interaction with dsDNA than ssDNA and more TB was concentrated on the dsDNA/MAA/Au surface, so the great increase of redox peak currents appeared. The different electrochemical responses of TB on ssDNA/MAA/Au and dsDNA/MAA/Au electrode indicated that TB was a good electrochemical indicator to distinguish the ssDNA or dsDNA on the surface of modified electrode.

Electrochemical Parameters of TB on DNA Modified Electrodes

With the increase of scan rate, both the reductive and oxidative peak currents increased in the range from 50 to 800 mV s^{-1}. The relationships of $\log I_{pc}$ *vs*. $\log\upsilon$ at ssDNA/MAA/Au and dsDNA/MAA/Au electrode were plotted, respectively. Two lines were observed with the slope values as 0.72 and 0.79, which were between the ideal value of diffusion-controlled process (0.5) and surface-controlled process (1.0), respectively. So the electrode process of TB on the modified electrodes was the combination of diffusion-controlled and surface-controlled. The contribution of surface-controlled process on dsDNA modified electrode was more than that on ssDNA modified electrode, which was due to the fact that more TB was bound to dsDNA on the surface of dsDNA/MAA/Au electrode.

The influences of TB concentration on the electrochemical response at ssDNA/MAA/Au or dsDNA/MAA/Au electrode were further recorded. With the increase of the TB concentration, the redox peak currents increased and reached a maximum value on the DNA modified electrode, which exhibited an expected shape of Langumuir adsorption behavior. According to the Langumuir adsorption thermodynamic equation [19]:

$$\frac{C}{I_p} = \frac{1}{KI_{p,\max}} + \frac{C}{I_{p,\max}}$$

where I_p represents the anodic or cathodic peak current, $I_{p,\max}$ the maximum peak current, C the concentration of TB and *K* the adsorption constant of TB on the DNA modified electrode. From the slope and the intercept of the plot of C/I_p *vs*. C, the values of $I_{p,max}$ and *K* can be obtained. So the relationships of C/I_p *vs*. C at ssDNA/MAA/Au and dsDNA/MAA/Au were constructed, respectively, with two straight lines observed. The values of *K* were calculated as 2.1×10^4 mol^{-1} and 2.4×10^4 mol^{-1}, respectively, which indicated that TB showed high absorption ability on the dsDNA/MAA/Au than on the ssDNA/MAA/Au electrode.

The electron transfer kinetics parameters of TB at different electrodes were further calculated by the Laviron's equation [25]:

$$E_p = E^0 + \frac{RT}{\alpha nF}[\ln(\frac{k_s RT}{\alpha nF})] - \frac{RT}{\alpha nF}\ln\upsilon$$

where E_p represents the redox peak potential, E^0 the standard electrode potential, R the gas constant, T the absolute temperature, α the electron transfer coefficient, n the number of electrons transferred in the rate determining reaction, F the Faraday constant, and k_s the electrode reaction standard rate constant. By exploring the relationship of E_p with lnυ, three lines were got. Then the values of α and k_s were calculated as 0.42, 0.43, 0.49 and 18.3, 19.3, 20.6 cm s^{-1}, respectively, for Au, ssDNA/MAA/Au and dsDNA/MAA/Au electrode.

The results indicated a typical surface-controlled quasi-reversible process. Also the results indicated that the electron transfer rate of TB at dsDNA/MAA/Au surface was faster than that on bare gold and ssDNA/MAA/Au electrode. Ju *et al*. had applied different electrochemical indicators such as nile blue [19], methylene blue [26] and di(2,2'-bipyridine) osmium(III) [27] in the electrochemical DNA biosensor. The electrochemical parameters of these electrochemical biosensors were calculated by the similar methods used in this paper. The k_s value with the nile blue as the indicator was smaller than the result of this work, indicating that TB had a high electron transfer rate on the DNA modified electrode.

Optimal of TB Concentration and Reaction Time

The TB concentration will influence the detection sensitivity of the DNA biosensor. With the increase of TB concentration, the cathodic peak current increased and reached a constant value at the TB concentration of 1.5×10^{-4} M, which indicated that TB was saturately adsorbed on the surface of DNA modified electrode. So a 1.5×10^{-4} M TB was selected in the following experiments.

The equilibrium time of TB on bare gold, ssDNA/MAA/Au and dsDNA/MAA/Au was investigated with the results shown in Fig. 2. The equilibrium time on different modified electrodes was 4 min, 8 min and 10 min, respectively indicating that more time was spent for TB to accumulate on the dsDNA modified electrode saturated to form a stable complex.

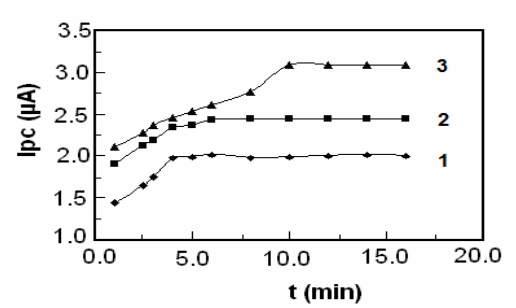

Fig. 2. Influence of TB reaction time on the peak current with bare Au (1), ssDNA/MAA/Au (2) and dsDNA/MAA /Au (3) electrode.

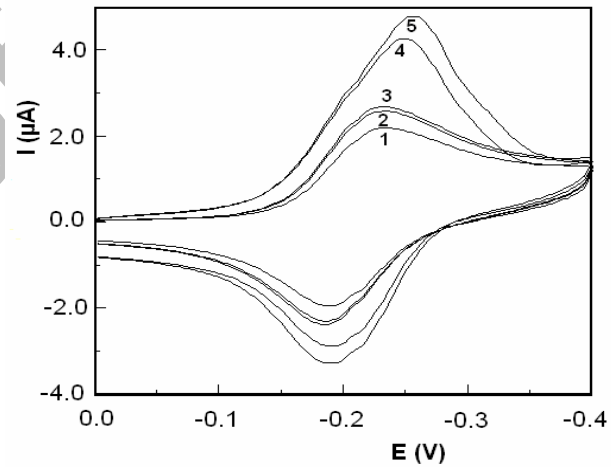

Fig. 3. Cyclic votlammograms of 1.5×10^{-4} M TB on different DNA modified electrodes. (1) bare Au electrode; (2) actA probe/MAA/Au; (3) after hybridization with noncomplementary ssDNA; (4) after hybridization with two-base mismatch ssDNA; (5) after hybridization with actA target ssDNA.

Electrochemical Behavior of TB on the Detection of Hybridization with Synthetic Targets

Figure 3 shows the cyclic voltammograms of TB on the detection of hybridization process. Curve 1 is the cyclic voltammogram of TB on bare gold electrode with a pair of redox peaks. Curve 2 is that of TB on actA ssDNA probe modified electrode, a little increase of peak current was observed indicating that TB could interact with ssDNA on the surface of gold electrode. Curve 3 is that of the actA probe interacting with noncomplementary ssDNA sequence, the little increase of peak current may be caused by the non-specific adsorption of small amounts of ssDNA on the electrode surface resulting in the greater accumulation of TB, and the little increase of the peak current. Curve 5 is hybrid electrode with complementary target, a significant increase of TB signal was observed, which was due to the formation of dsDNA on the electrode surface, and more TB was accumulated on the electrode surface. Since TB had stronger interaction ability with dsDNA than ssDNA, so a big electrochemical response appeared. Curve 4 is the hybridization of probe with a two-base mismatch ssDNA sequence. Compared with that of curve 5, a little decrease of peak current appeared, which

indicates that the hybridization with the two-base mismatch ssDNA resulted in a partially formed hybrid, so the amounts of TB on the electrode surface was smaller than that on the dsDNA modified electrode, and the decrease of TB electrochemical signal appeared. The above results indicate that the actA electrochemical DNA biosensor displayed a higher selectivity for the hybridization and could be successfully applied to the discrimination of specific sequences.

Detection of Synthetic actA Sequence

The actA ssDNA probe modified electrode was further applied to the detection of synthetic target actA sequences with the results shown in Fig. 4. The reductive peak current of TB was increased linearly with the target ssDNA sequence in the concentration range from 1.0×10^{-7} to 8.0×10^{-5} M with the linear regression equation as ΔI_{pc} (μA) = 0.562logC (M) + 3.97 (n = 6, R = 0.994), where ΔI_{pc} was the reduction peak current difference of TB before and after the hybridization. The detection limit was calculated as 4.60×10^{-8} M (3σ), where σ is the relative standard deviation (R.S.D.) of the blank solution (n = 7) (Fig. 4).

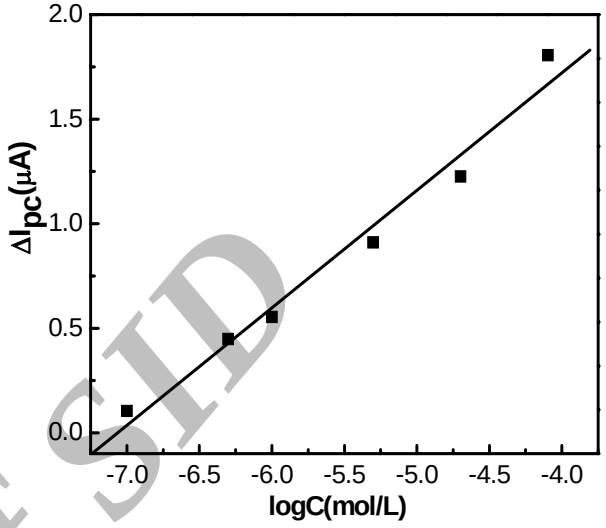

Fig. 4. Linear relationship between ΔI_{pc} and logarithms of target ssDNA sequence concentrations.

Application of Electrochemical DNA Biosensor for the PCR Product

Figure 5 shows the images of the PCR amplification specific for an actA gene. It can be seen that the PCR procedure was successfully performed and the fragments were about 222 pb after the amplification.

The proposed electrochemical DNA biosensor was directly applied to the detection of PCR product of actA gene samples. The PCR product of actA gene was diluted with 5.0 mM Tris-HCl buffer and denatured by heating it in boiling water for 5 min and then frozen in an ice bath for 2 min. The primer 1 of actA gene was immobilized on the MAA/Au with the established method to obtain an actA ssDNA/MAA/Au electrode. Then 10 μl of denatured PCR products was dipped on the surface of actA ssDNA/MAA/Au electrode and the hybridization reaction took place at 25 ºC for 60 min. After the hybridization reaction the electrochemical detection was applied using the general procedure and the detection results are shown in Fig. 6. The TB signal at the bare gold electrode (curve 1) is lower than that at the primer 1 ssDNA/MAA/Au electrode (curve 2). An obvious increase of the TB signal appeared after the hybridization of the probe with real sample PCR products (curve 3). This significant difference of the TB redox signals on the actA ssDNA/MAA/Au electrode and the hybridized dsDNA/MAA/Au electrode indicate that the electrochemical DNA biosensor could recognize and detect the PCR amplified product effectively.

CONCLUSIONS

In this paper TB was selected as an electrochemical indicator for the DNA electrochemical biosensor, which showed different redox behaviors on the ssDNA and dsDNA modified electrodes. The electrochemical parameters of TB on DNA modified electrodes were calculated with the related electrochemical parameters calculated. The proposed electrochemical DNA biosensor was successfully applied to the detection of specific sequences from actA gene with the dynamic range from 1.0×10^{-7} to 8.0×10^{-5} M. The PCR products of actA gene from *Listeria monocytogenes* was satisfactorily detected with this method, which extended the electrochemcial DNA biosensor to the detection of the foodborne pathogens.

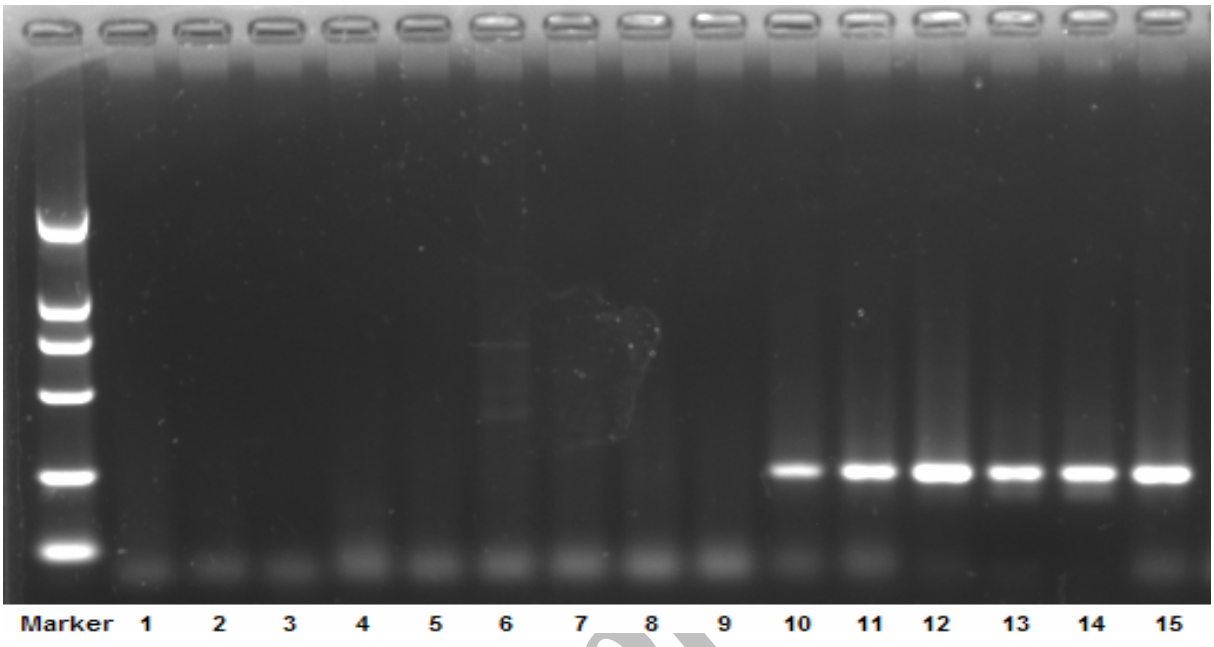

Fig. 5. The PCR amplification specific for actA gene of *Listeria monocytogenes*: Lane 1, *Listeria ivanovii*; Lane 2, *Listeria grayi*; Lane 3, *Listeria seeligeri*; Lane 4, *Listeria innocua*; Lane 5, *Listeria welshimeri*; Lane 6, *Staphylococcus aureus*; Lane 7, *Yersinia enterorcolitica*; Lane 8, *Salmonella aberdeen*; Lane 9, *Shigella flexneri*; Lanes 10-15, *6 Listeria monocytogenes* M: DL 2000 (Takara, China) was used as molecular weight marker; fragments from top to bottom: 2000, 1000, 750, 500, 250 and 100 base pairs.

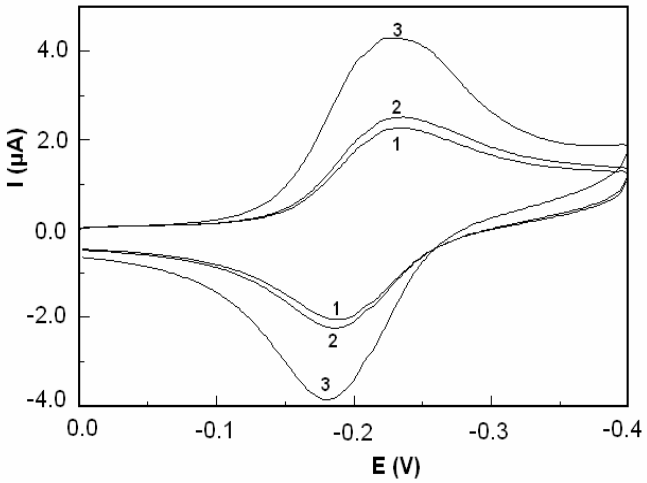

Fig. 6. Cyclic voltammograms for the detection of PCR Samples by using 1.5×10^{-4} M TB at bare gold electrode (1), actA probe/MAA/Au electrode (2), the electrode hybridization with PCR amplified real sample (3).

ACKNOWLEDGEMENTS

We are grateful to the financial support of Major State Basic Research Development Program of China (2006BAD05A06-01), Research Project of the General Administration of Quality Supervision, Inspection and Quarantine of P.R. China, and Shandong Province Natural Science Foundation (2009ZRB01299).

REFERENCES

[1] J. Wang, Anal. Chim. Acta 469 (2002) 63.
[2] T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechnol. 21 (2003) 1192.
[3] J.F. Rusling, Acc. Chem. Res. 31 (1998) 363.
[4] R.E. Sabzi, B. Sehatnia, M.H. Pournaghi-Azar, M.S. Hejazi, J. Iran. Chem. Soc. 5 (2008) 476.
[5] W. Sun, J.H. Zhong, P. Qin, K. Jiao, Anal. Biochem.

377 (2008) 115.
[6] S. Reisberg, L.A. Dang, Q.A. Nguyen, B. Piro, V. Noel, P.E. Nielsen, L.A. Le, M.C. Pham, Talanta 76 (2008) 206.
[7] F. Lucarelli, G. Marrazza, A.P.F. Turner, M. Mascini, Biosens. Bioelectron. 19 (2004) 515
[8] J. Wang, Anal. Chim. Acta 500 (2003) 247.
[9] C. Jiang, T. Yang, K. Jiao, H.G. Wei, Electrochim. Acta 53 (2008) 2917.
[10] H.Y. Ma, L.P. Zhang, Y. Pan, K.Y. Zhang, Y.Z. Zhang, Electroanalysis 20 (2008) 1220.
[11] J. Wang, M. Chicharro, G. Rivas, X.H. Cai, N. Dontha, P.A.M. Farias, H. Shiraishi, Anal. Chem. 68 (1996) 2251.
[12] S.H. Liu, C.L. Sun, P.G. He, Y.Z. Fang, Chinese J. Anal. Chem. 27 (1999) 131.
[13] B.Z. Wang, X.Y. Du, J. Zheng, B.F. Jin, Chinese J. Anal. Chem. 33 (2005) 789.
[14] K. Kerman, D. Ozkan, P. Kara, B. Meric, J.J. Gooding, M. Ozsoz, Anal. Chim. Acta 462 (2002) 39.
[15] M. Gao, H.L. Qi, Q. Gao, C.X. Zhang, Electroanalysis 20 (2008) 123.
[16] A. Erdem, K. Kerman, B. Meric, M. Ozsoz, Electroanalysis 13 (2001) 219.
[17] K.M. Millan, S.R. Mikkelsen, Anal. Chem. 65 (1993) 2317.
[18] M.I. Pividori, A. Merkoçi, S. Alegret, Biosens. Bioelectron. 16 (2001) 1133.
[19] H.X. Ju, Y.K. Ye, Y.L. Zhu, Electrochim. Acta 50 (2005) 1361.
[20] P.S. Mead, L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, R.V. Tauxe, Emerg. Infect. Dis. 5 (1999) 607.
[21] L.J. Passmore, A.A. Killeen, Mol. Diagn. 1 (1996) 329.
[22] W. Sun, N. Zhao, X. Xu, K. Jiao, Chinese J. Anal. Chem. 35 (2007) 1548.
[23] K. Jiao, Q.J. Li, W. Sun, Z.J. Wang, Electroanalysis 17 (2005) 997.
[24] A. Malinauskas, T. Ruzgas, L. Gorton, J. Solid State Electrochem. 5 (2008) 287.
[25] E. Laviron, J. Electroanal. Chem. 101 (1979) 19.
[26] J.Y. Gu, X.J. Lu, H.X. Ju, Electroanalysis 14 (2002) 949.
[27] H.X. Ju, Y.K. Ye, J.H. Zhao, Y.L. Zhu, Anal. Biochem. 313 (2003) 255.