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Rapid and Sensitive Strategy for *Salmonella* Detection Using an *InvA* Gene-Based Electrochemical DNA Sensor

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A rapid and sensitive strategy for the detection of *Salmonella* was proposed by integrating simple DNA extraction, specific polymerase chain reaction (PCR) with an *invA* gene-based electrochemical DNA sensor. The amplified target sequence of *invA* gene could be specifically captured on the sensing interface, and further hybridized with biotinylated detection probe to form a sandwich-type hybridization structure. The electrochemical signal was amplified by streptavidin-alkaline phosphatase (ST-AP), producing sensitive enzyme-catalyzed electrochemical DNA sensing. The fabrication and hybridization processes were characterized with electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV) and surface plasmon resonance (SPR). The designed DNA sensor could discriminate satisfactorily the complementary and mismatched oligonucleotides, indicating good selectivity. The linear calibration range for target DNA detection was from 1 pM to 10 nM with a detection limit of 0.5 pM, showing high sensitivity. Under optimal conditions, the proposed strategy could quantitatively detect *Salmonella* from 10 to 10^5 CFU mL^{-1} within 3.5 h. This strategy presented a simple, rapid and sensitive platform for *Salmonella* detection and would become a powerful tool for pathogenic microorganisms screening in clinical diagnostics, food safety, biothreat detection and environmental monitoring.

Keywords: Biosensor, Electrochemistry, Polymerase chain reaction, Salmonella, InvA gene

1. INTRODUCTION

Salmonella, as one of the most common pathogens of foodborne disease worldwide [1], is responsible for a large number of infections in both humans and animals [2]. It is estimated that *Salmonella* causes 93.8 million human infections and 155,000 deaths annually worldwide [3]. Therefore, sensitive and rapid detection of *Salmonella* is of out-most importance in the field of food safety, biothreat prevention and public health.

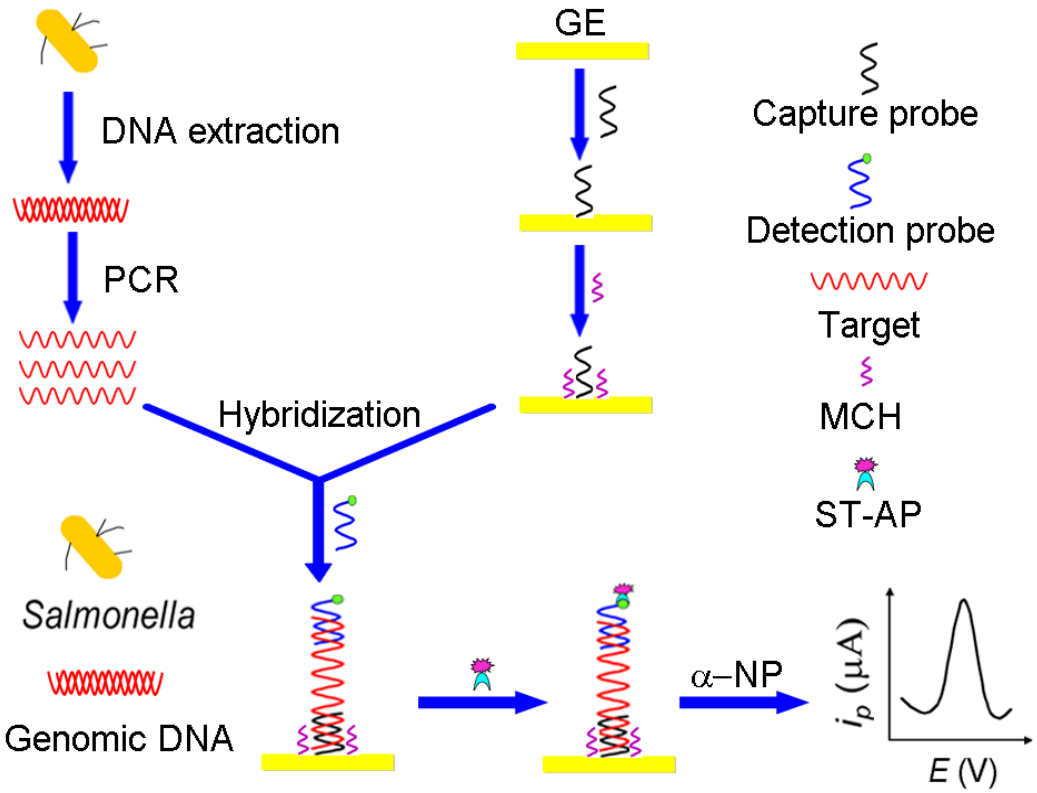
Various methods have been used for the detection of *Salmonella*, including conventional culture methods, enzyme-linked immunosorbant assay (ELISA) and real-time quantitative polymerase chain reaction (RT-PCR). Conventional culture methods are reliable, but time consuming and quite laborious [4]. ELISA usually requires a minimum time of 24-48 h, and its sensitivity ($\geq 10^5$ CFU mL^{-1}) is insufficient to detect low levels of pathogen [5]. PCR method has distinct advantages in sensitivity [6], but it often encounters false positivity [7], and the low resolution of post-PCR analysis by gel electrophoresis also limits its routine use in many laboratories.

In comparison to traditional PCR, real-time quantitative PCR has a large dynamic range, increased sensitivity, and can be highly sequence-specific [8], however, it requires expensive, specialized equipment and highly trained personnel [9]. Recently, considerable interest has focused on developing simple and sensitive biosensing methods for rapid detection of *salmonella*, including SPR [10], field effect transistor (FET) [11], fluorescence [1], magnetoelastic biosensor [12], capacitive immunosensor [13], quartz crystal microbalance (QCM) [14], fiber-optic biosensor, immunosensor based on electrical impedance techiniques [15], piezoelectric immunosensor [16], and electrochemical biosensor [17].

Among the available biosensor platforms, electrochemical approach has received remarkable attention due to its high sensitivity, fast response, low cost and suitability to miniaturization [18, 19]. In this work, in order to overcome the inherent disadvantages of traditional PCR-based method and implement sensitive and rapid screening of pathogens, a simple strategy for detection of *Salmonella* was developed by integrating rapid DNA extraction, specific PCR with an *invA* gene-based electrochemical DNA sensor.

The *invA* target gene is located on *Salmonella* pathogenicity island 1 (SPI-1), which is essential for the invasion of epithelial cells by *Salmonella* [20]. This gene is highly conserved in almost all *Salmonella* serotypes [21] and has been used as a potential target for *Salmonella* detection [22, 23]. To our knowledge, no *invA* gene-based electrochemical DNA sensor has been reported for the quantitative analysis of viable *Salmonella* cells. In this work, PCR primers and probes were specifically designed according to the *invA* gene sequence.

Genome DNA was extracted from *Salmonella* by a rapid and efficient boiling method, and PCR amplification was then performed with a pair of *invA* gene-specific primers. The denatured PCR product was captured at the electrode surface by sandwich hybridization with thiolated capture probe and biotinylated detection probe. ST-AP was then bound to biotinylated probe to catalyze the hydrolysis of α-naphthyl phosphate (α-NP), leading to enzymatic signal amplification for sensitive detection of target DNA sequence (Scheme 1). This work provided an applicable strategy for detection of *Salmonella.*



Scheme 1. Schematic illustration of the strategy for *Salmonella* detection using an *invA* gene-based electrochemical DNA sensor.

2. EXPERIMENTAL

2.1. Reagents

6-mercapto-1-hexanol (MCH), ST-AP, α -NP, bovine serum albumin (BSA) and salmon sperm DNA were obtained from Sigma-Aldrich. Premix Taq Version 2.0, DL500 DNA Marker and agarose were purchased from Takara (Dalian, China). All other reagents were of analytical grade. All solutions were prepared using Millipore-Q water (≥18 MΩ).

2.2. Oligonucleotides

The *invA* gene was used to design specific probes for *Salmonella* by exploring the Gene Bank database. The specificity of primers for the PCR amplification of *invA* gene fragment and probes for DNA sensing had been positively verified via the BLAST search engine (http://www.ncbi.nlm.nih.gov/blast). Oligonucleotides with the sequences shown in Table 1 were synthesized by Invitrogen (Shanghai, China). All oligonucleotides were dissolved in tris-ethylenediaminetetraacetic acid (TE) buffer (10 mM Tris-HCl, 1mM ethylenediaminetetraacetic acid; pH 8.0) and stored at -20 °C, which were diluted in appropriate buffer prior to use.

Table 1. Oligonucleotides used in the present work.

Oligonucleotide	Sequence (5'-3')
Forward primer	TCATCGCACCGTCAAAGGAACC
Reverse primer	GTGAAATTATCGCCACGTTCGGGCAA
Capture probe	HS-$(CH_2)_6$-CGCACCGTCAAAGGAA
Detection probe	TACCGGCCTTCAAATCGGCA-biotin
Target oligonucleotide	TGCCGATTTGAAGGCCGGTAGCTAGATTCCTTTGACGGTGCG
Single-base-mismatched oligonucleotide	TGCCGATTTGAAGGCCGGTAGCTAGATTCCTTTGGCGGTGCG
Non-complementary oligonucleotide	ATGGGCTATAGCTGCATGCGTTACGAGCTGGGAGCGAGTAGC

2.3. Apparatus

All electrochemical measurements were performed on a CHI660D electrochemical workstation (Shanghai Chenhua Instruments Co. Ltd., China) with a conventional three-electrode system composed of platinum wire as auxiliary, Ag/AgCl electrode as reference, and a 3-mm-diameter gold electrode (GE) as working electrode. The PCR reaction was carried out using a My Cycler thermal cycler (Bio-Rad Laboratories, USA). Gel images were captured on an imaging system (Bio-Rad Laboratories, USA). SPR analysis was completed on BIACORE X™ instrument (Biacore AB, Uppsala, Sweden).

2.4. Preparation of DNA samples

Salmonella typhimurium strains were grown aerobically at 37 °C for 16 h in Luria-Bertani medium. Viable counts were performed by plating 100 μL of appropriate 10-fold dilutions in sterile phosphate buffer saline (PBS) onto plate count agar in triplicate and incubating the plate for 24 h at 37 °C. The concentration was estimated by calculating the average number of CFU. One milliliter aliquot of each bacterial culture was centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was carefully removed and the cell pellet was washed in 1 mL water. After centrifugation, the cell pellet was resuspended in 100 μL water. The microcentrifuge tube was incubated for 15 min at 100 °C in a water bath and immediately chilled on ice. After centrifugation at 10,000 rpm for 5 min at 4 °C, the supernatant containing genome DNA was carefully transferred to a new tube. A 5 μL aliquot was used as template DNA for the PCR. All DNA preparations were stored at -20 °C prior to use.

2.5. PCR amplification

The reaction mixture in a final volume of 50 μL contained 5.0 μL of genomic DNA, 1.0 μL 20 μM of each primer, 25 μL of Premix Taq (1.25 U of DNA polymerase, 2 × Taq buffer, 0.4 mM of dNTPs) and 18 μL of water. After denaturing at 95 °C for 1 min, the reaction was carried out for 35

cycles of 95 °C for 30 s, 64 °C for 30 s, and 72 °C for 30 s. A final extension of 72 °C for 4 min was employed. PCR products were determined by running 10 μL of PCR mixture in 2% agarose gel for 20 min and observed under ultraviolet light.

2.6. Preparation of DNA sensor

A bare GE was polished with 0.05 μm alumina slurries and ultrasonically treated in ultrapure water, followed by soaking in piranha solution (H_2SO_4 : H_2O_2 = 3 : 1) for 10min to eliminate other substances. 10 μL of 1.0 μM thiolated capture probe was dropped onto the pretreated electrode surface and incubated overnight at 4 °C. After washing with 0.1 M pH 8.0 Tris-HCl containing 0.05% Tween-20, the electrode was immersed into 100 μL of 1 mM MCH solution for 1 h to obtain well-aligned DNA monolayer. The electrode was further treated with blocking agent solution (125 μg mL^{-1} salmon sperm DNA and 2% BSA in 0.1 M pH 8.0 Tris-HCl) to avoid nonspecific adsorption of DNA and enzyme on the electrode surface and then thoroughly washed with 0.1 M Tris-HCl buffer containing 0.05% Tween-20.

2.7. Electrochemical detection of synthetic target oligonucleotides and PCR product

PCR product was denatured by heating for 5 min at 100 °C in a water bath, and immediately chilled in ice for 5 min to obtain denatured ssDNA before the detection. Both the synthetic target oligonucleotide and denatured PCR product were diluted to the desired concentration with 2 × Sodium chloride-sodium citrate buffer (2 × SSC, 0.3 M NaCl and 0.03 M sodium citrate, pH 8.0), respectively. A 10 μL aliquot of hybridization solution containing target oligonucleotide and 100 nM of biotinylated detection probe were dropped onto DNA sensor and incubated for 1 h at 37 °C to form a sandwich-type DNA hybridization. The sensor was then rinsed with diethanolamine buffer (0.1 M diethanolamine, 1 mM $MgCl_2$, 100 mM KCl, pH 9.6) containing 0.05% Tween-20 thoroughly.

After the hybridization step, 10 μL of diethanolamine buffer containing 1.25 mg mL^{-1} of ST-AP and 10 mg mL^{-1} of BSA was dropped onto sensor surface. After 30 min of incubation, the sensor was washed with diethanolamine buffer containing 0.05% Tween-20 thoroughly. The electrochemical measurement was performed in diethanolamine buffer containing 1 mg mL^{-1} of α-NP. The electrochemical oxidation signal of the enzymatically-produced α-naphthol was measured by differential pulse voltammetry (DPV) (modulation time = 0.05 s; interval time = 0.017 s; step potential = 5 mV; modulation amplitude = 70 mV; potential scan: from 0.0 to +0.6 V).

3. RESULTS AND DISCUSSION

3.1. Characterization of DNA sensor

EIS is a powerful tool to monitor the whole procedure in preparing modified electrodes and provide useful information on various properties including the electrode impedance, the capacity of the

electric double layer, and the surface electron transfer resistance (Ret) of the electrode surface in different modification stages [24]. Figure 1A shows the Nyquist plots of $Fe(CN)_6^{3-/4-}$ containing 0.4 M KCl at different electrodes. The bare GE exhibits an almost straight line that is characteristic of a diffusional limiting electron-transfer process (curve a). The immobilization of thiol-modified capture probe and MCH results in a remarkably increased Ret (curve b). This can be attributed to physical coverage by the oligonucleotides and repulsive electrostatic interaction between negatively charged phosphate backbone of the single strand nucleic acid and ferricyanide anion [25]. MCH was employed to force the tethered DNA strands "stand up" on the electrode surface and reduce its nonspecific adsorption on the surface through hydrophobic and electrostatic interaction [26], led to the further increase of Ret. The Ret increased again after the sensor was incubated with target oligonucleotide and detection probe (curve c) due to the increase of the negative charges after hybridization, indicating successful achievement of sandwich-type DNA hybridization. These results were in a good agreement with those obtained from SWV (Figure 1B), in which the peak current decreased upon the assembly and hybridization processes.

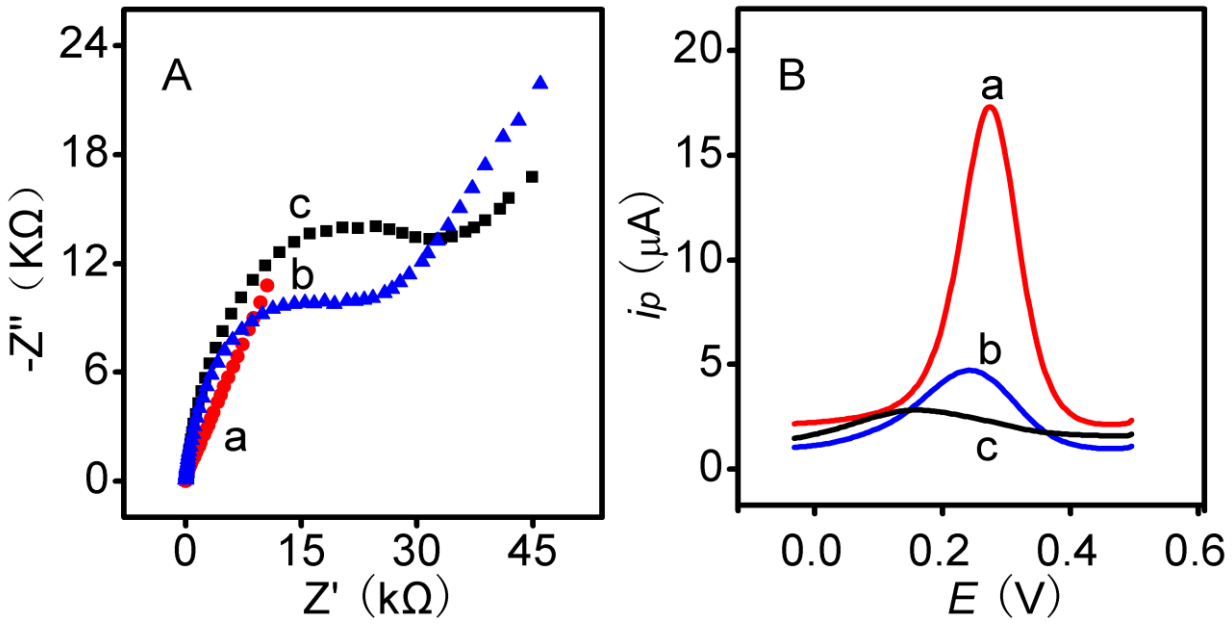


Figure 1. EIS (A) and SWVs (B) of bare electrode (a), capture probe modified electrode after exposure to MCH solution (b), capture probe and MCH modified electrode after hybridization with 5 nM target DNA for 1 h at 37°C (c) in 0.5 mM $Fe(CN)_6^{3-/4-}$ containing 0.4 M KCl.

The stepwise reactions on the DNA sensor were also characterized by SPR (Figure 2). A bare gold chip was firstly modified with capture probe and then treated with MCH. The SPR response showed remarkable increase of resonance unit (RU) after hybridization with target DNA and biotinylated detection probe. Upon further binding with the ST-AP to the chip surface, a considerable increase in RU was observed. Thus it could be concluded that the processes of assembly, hybridization and the binding with ST-AP were successfully achieved on the sensor surface.

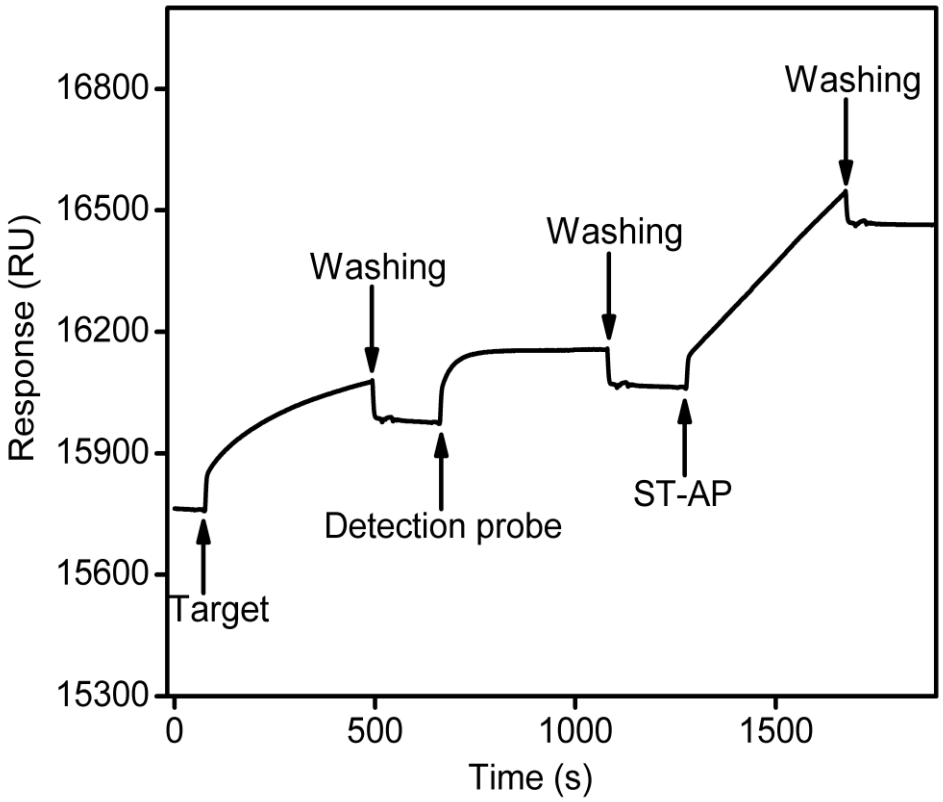


Figure 2. Characterization of sensor fabrication by SPR.

3.2. Optimization of experimental conditions

The incubation time for sandwich-type DNA hybridization was optimized. With the increasing incubation time, the DPV peak current sharply increased and tended to a steady value after 60 min (Figure 3A). To shorten the analysis time, 60 min was chosen as the optimal incubation time for DNA hybridization.

The effect of ST-AP concentration and incubation time for binding with biotinylated detection probe after hybridization reaction on the DPV response was investigated (Figure 3B, 3C). The DPV signal sharply increased with the increasing ST-AP concentration and reached a plateau after 1.25 μg mL^{-1} due to the saturated binding. Further increase in ST-AP concentration would increase its nonspecific adsorption on sensor surface. With the increasing incubation time of ST-AP, the DPV peak current also sharply increased and tended to a steady value after 30 min. Thus, 1.25 μg mL^{-1} ST-AP and the incubation of 30 min were chosen as the optimal conditions for ST-AP binding.

The performance of the electrochemical analysis was related to the concentration of α-NP in the measuring system. The DPV peak current of the DNA sensor increased with the increasing concentration of α-NP from 0 to 1 mg mL^{-1}, and then maintained the maximum value at higher concentrations. After all, the enzymatic reaction rate depended on the amount of the ST-AP bound on the sensor surface. Therefore, the optimal α-NP concentration for DPV detection was 1 mg mL^{-1} (Figure 3D).

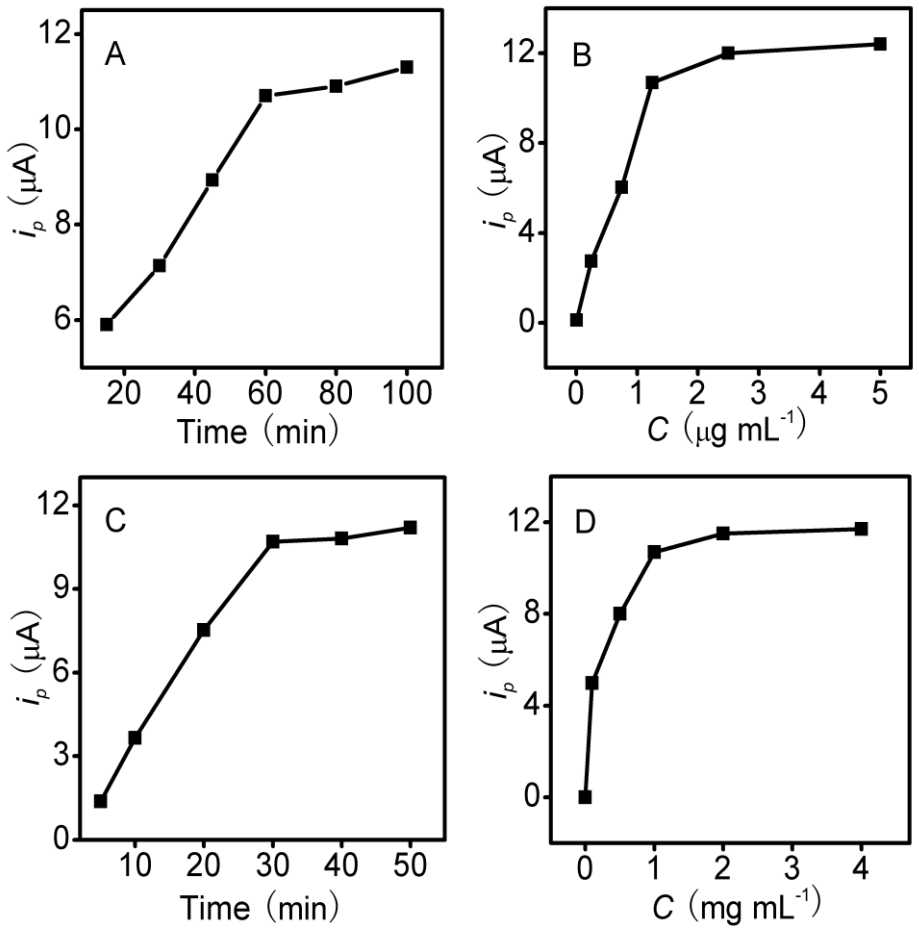


Figure 3. Dependences of DPV peak currents on hybridization time in 5 nM target DNA (A), ST-AP concentration (B), incubation time in ST-AP (C), and α-NP concentration (D). When one parameter changes others are under their optimal conditions.

3.3. Specificity of the strategy

In this work, PCR primers and probes were specifically designed according to the *invA* gene sequence, the specificity of *invA* primers has been verified, non-*Salmonella* strains did not amplify and no nonspecific products were amplified [20], so the specificity of the proposed strategy was evaluated only by investigating the selectivity of DNA sensor for oligonucleotides. Fully complementary oligonucleotides, single-base-mismatched oligonucleotides and non-complementary oligonucleotides were analyzed under the same optimized conditions, DPV responses of the DNA sensor were compared after hybridization with 5 nM and 100 pM of the three different oligonucleotides (Figure 4), respectively. Although the DNA sensor could respond to the single-base mismatched sequence, both the responses were significantly weaker than those of the complementary sequences at the two concentrations. The DPV responses with the non-complementary oligonucleotides were very poor. These results demonstrated that the designed DNA sensor could discriminate different DNA sequences effectively and displayed excellent selectivity. Thus, high specificity of the proposed strategy for

detection of *Salmonella* is ensured through PCR amplification combined with an *invA* gene-based electrochemical DNA sensor.

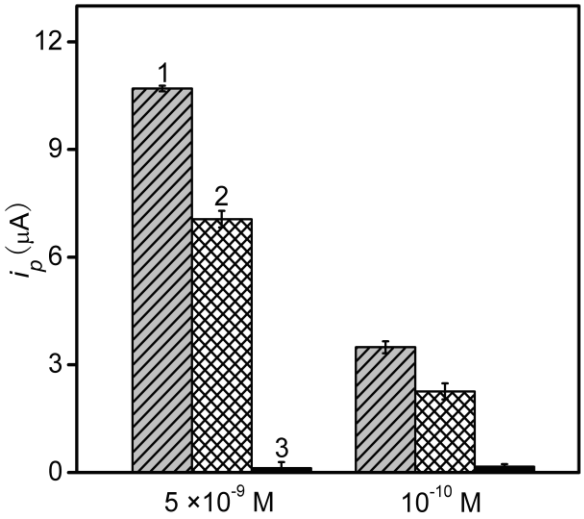


Figure 4. Comparison of DPV peak currents after hybridization with 5 nM and 100 pM of target oligonucleotides (1), single-base-mismatched oligonucleotides (2), non-complementary oligonucleotides (3).

3.4. Analytical performance of DNA sensor

To elucidate the analytical performance of the designed DNA sensor, synthetic target oligonucleotides with different concentrations were analyzed (Figure 5A).

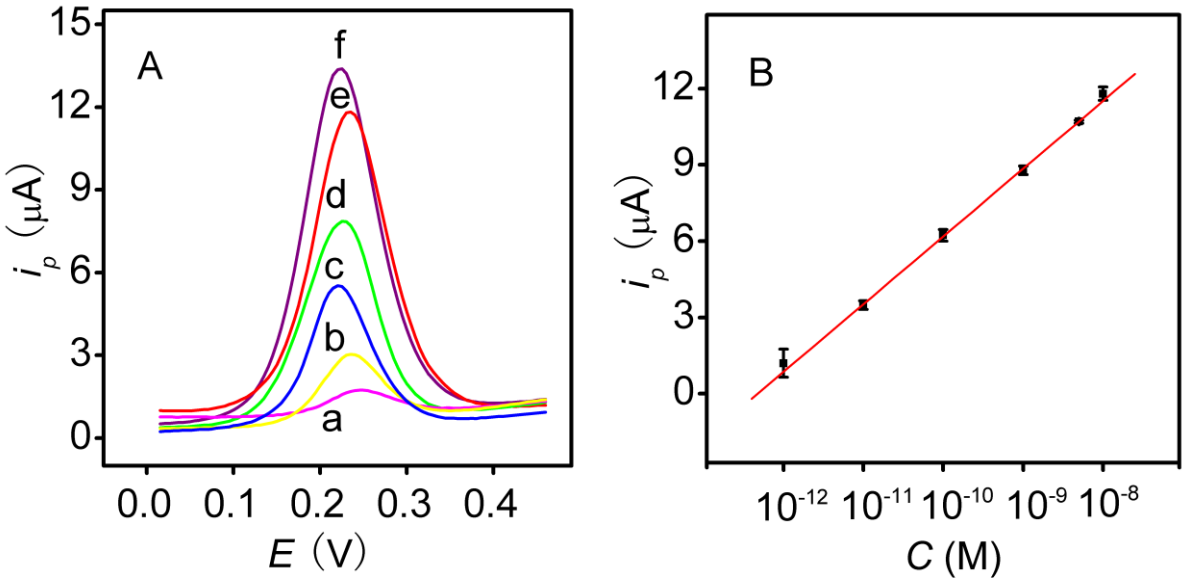


Figure 5. (A) DPV curves of the sensor obtained with target DNA concentrations of 1.0, 10, 100 pM, 1.0, 5.0 and 10 nM (from a to f). (B) Plot of DPV peak current *vs* target DNA concentration.

Under the optimal experimental conditions, the DPV response was linear with the logarithm of target DNA concentration in the range from 1.0 pM to 10 nM. The corresponding regression equation was i_p (μA) = 32.82 + 2.66 × lg C (M) with the correlation coefficient of 0.9996 (Figure 5B). The limit of detection (LOD), which is defined as three times the standard deviation of the blank sample measurements, is estimated to be 0.5 pM. The low LOD of the DNA sensor was achieved due to low nonspecific absorption on the electrode surface, the strong binding of streptavidin-biotin, and efficient signal amplification through catalytic generation of a large number of alkaline phosphatase.

The reproducibility of the proposed sensor was investigated by detecting synthetic target DNA at 5 nM and 100 pM with six replicates, respectively. Relative standard deviations (RSD) for both concentrations were less than 5%, which indicated a satisfactory reproducibility of the designed DNA sensor.

3.5. Detection of Salmonella

PCR was performed using the genomic DNA extracted from *Salmonella* with a series of concentrations.

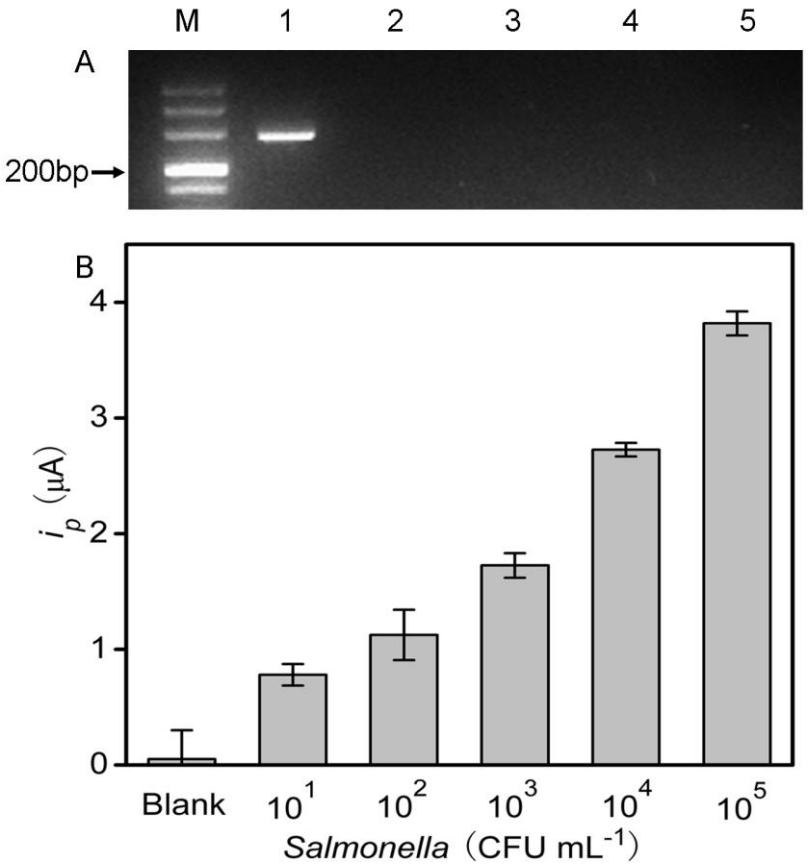


Figure 6. (A) Gel electrophoresis photos of 500 bp size maker (M), and PCR products of 10^8 (1), 10^1 (2), 10^2 (3), 10^3 (4), 10^4 (5) CFU mL^{-1} *Salmonella*. (B) DPV peak currents responding to PCR products obtained from serial dilutions of *Salmonella* in the range of 10-10^5 CFU mL^{-1}.

The amplification of a 284 bp fragment of *invA* gene was successfully achieved, which could be verified by 2 % agarose gel electrophoresis (Fig. 6A, line 1). However, no target band could be observed in PCR products corresponding to 10-10^4 CFU mL^{-1} of *Salmonella* due to the low resolution of gel electrophoresis (Figure 6A, line 2-5). Then the electrochemical DNA sensor was applied to analyze the denatured PCR products, DPV peak current was proportional with the concentration of *Salmonella* over the range of 10-10^5 CFU mL^{-1} (Figure 6B). The sensitivity of the proposed strategy was compared with those of other methods reported previously for the detection of *Salmonella* (Table 2), our method can detect at least 10 CFU mL^{-1} of *Salmonella*, shows higher sensitivity than other current techniques and makes it possible to implement convenient detection of *Salmonella* at extremely low concentration without enrichment. Furthermore, the proposed method can shorten the detection time substantially from one week in traditional method to 3.5 h. These results indicated that the proposed strategy is sensitive, rapid, and considerably simpler than traditional methods for *Salmonella* detection due to the integration of a simple DNA extraction, specific PCR with a high sensitive electrochemical DNA sensor for *invA* gene. Future work will be focused on the optimization of a standardized sample preparation procedure and the strategy could be potentially developed as a pragmatic tool for *Salmonella* monitoring in real samples. Moreover, the methodology can easily be extended to other pathogens by the use of appropriate oligonucleotides.

Table 2. Comparison between the proposed method and other reported biosensors for the detection of *Salmonella*.

Biosensor Platform	Bio-receptor of immobilisation	LOD (CFU mL^{-1})	Ref.
SPR	Antibody	5×10^6	[10]
FET	Antibody	10^2	[11]
Fluorescence	Oligonucleotide	30	[1]
Magnetoelastic	E2 phage	5×10^2	[12]
Capacitive	Antibody	10^2	[13]
QCM	Antibody	10^2	[14]
Fiber-Optic	Antibody	10^3	[15]
Electrical impedance	Antibody	10^3	[5]
Piezoelectric crystals	Antibody	10^5	[16]
Electrochemical(chronoamperometry)	Antibody	5×10^3	[17]
Electrochemical (DPV)	Oligonucleotide	10	This study

4. CONCLUSIONS

The work presented here describes the development of a novel strategy for sensitive and rapid detection of *Salmonella* by employing a simple DNA extraction, PCR amplification and an electrochemical DNA sensor. Amplification of a 284 bp fragment of *invA* gene specific for *Salmonella*

was confirmed by agarose gel electrophoresis. An enzymatic electrochemical sensor based on the highly specific DNA probes for *invA* gene sequence recognition was successfully developed and exhibited high sensitivity, satisfactory selectivity and good reproducibility. The applicability of the strategy was demonstrated by measuring low levels of *Salmonella* down to 10 cfu mL^{-1} in just 3.5 h. This proposed strategy possessed the advantages of excellent sensitivity, rapid detection and low cost, which would provide a powerful tool for *Salmonella* screening in biomedical diagnostics, food safety, biothreat detection and environmental monitoring.

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References

1. Z.P. Wang, H. Xu, J. Wu, J. Ye, Z. Yang, *Food Chem.*, 125 (2011) 779
2. C. Tirado, K. Schmidt, *J. Infect.*, 43 (2001) 80
3. R.S. Hendriksen, A.R. Vieira, S. Karlsmose, D.M.A. Lo Fo Wong, A.B. Jensen, H.C. Wegener, F.M. Aarestrup, *Foodborne Pathog. Dis.*, 8 (2011) 887
4. L. Cocolin, M. Manzano, C. Cantoni, G. Comi, *J. Appl. Microbiol.*, 85 (1998) 673
5. R. Dev Das, C. RoyChaudhuri, S. Maji, S. Das, H. Saha, *Biosens. Bioelectron.*, 24 (2009) 3215
6. R. García, J. Bælum, L. Fredslund, P. Santorum, C.S. Jacobsen, *Appl. Environ. Microbiol.*, 76 (2010) 5025
7. J.R. Johnson, *J. Microbiol. Meth.*, 41 (2000) 201
8. M.L. Wong, J.F. Medrano, *Biotechniques*, 39 (2005) 75
9. J. Klonoski, R. Mondesire, L. Rea, D.C. Ward, R.D. Jenison, *Anal. Biochem.*, 396 (2010) 284
10. D. Kyprianou, A.R. Guerreiro, M. Nirschl, I. Chianella, S. Subrahmanyan, A.P.F. Turner, S. Piletsky, *Biosens. Bioelectron.*, 25 (2010) 1049
11. R.A. Villamizar, A. Maroto, F.X. Rius, I. Inza, M.J. Figueras, *Biosens. Bioelectron.*, 24 (2008) 279
12. S.Q. Li, Y.G. Li, H.Q. Chen, S. Horikawa, W. Shen, A. Simonian, B.A. Chin, *Biosens. Bioelectron.*, 26 (2010) 1313
13. G.J. Yang, J.L. Huang, W.J. Meng, M. Shen, X.A. Jiao, *Anal. Chim. Acta*, 647 (2009) 159
14. S.T. Pathirana, J. Barbaree, B.A. Chin, M.G. Hartell, W.C. Neely, *Biosens. Bioelectron.*, 15 (2000) 135
15. A.M. Valadez, C.A. Lana, S.I. Tu, M.T. Morgan, A.K. Bhunia, *Sensors*, 9 (2009) 5810
16. S.H. Si, X. Li, Y.S. Fung, D.R. Zhu, *Microchem. J.*, 68 (2001) 21
17. F. Salam, I.E. Tothill, *Biosens. Bioelectron.*, 24 (2009) 2630
18. L. Tang, G.M. Zeng, G.L. Shen, Y.P. Li, C. Liu, Z. Li, J. Luo, C.Z. Fan, C.P. Yang, *Biosens. Bioelectron.*, 24 (2009) 1474
19. M. Tichoniuk, D. Gwiazdowska, M. Ligaj, M. Filipiak, *Biosens. Bioelectron.*, 26 (2010) 1618
20. B. Malorny, J. Hoorfar, C. Bunge, R. Helmuth, *Appl. Environ. Microbiol.*, 69 (2003) 290
21. L.T. Daum, W.J. Barnes, J.C. McAvin, M.S. Neidert, L.A. Cooper, W.B. Huff, L. Gaul, W.S. Riggins, S. Morris, A. Salmen, K.L. Lohman, *J. Clin. Microbiol.*, 40 (2002) 3050
22. K. Rahn, S.A. De Grandis, R.C. Clarke, S.A. McEwen, J.E. Galán, C. Ginocchio, R. Curtiss , C.L. Gyles, *Mol. Cell. Probes*, 6 (1992) 271
23. E.S. Jeong, K.S. Lee, S.H. Heo, J.H. Seo, Y.K. Choi, *Exp. Anim.*, 60 (2011) 65
24. S.J. Ye, H.X. Li, W. Cao, *Biosens. Bioelectron.*, 26 (2011) 2215

25. M. Yang, H.C.M Yau, H. L. Chan, *Langmuir*, 14 (1998) 6121
26. A. Singh, S.K. Arya, N. Glass, P. Hanifi-Moghaddam, R. Naidoo, C.M. Szymanski, J. Tanha, S. Evoy, *Biosens. Bioelectron.*, 26 (2010) 131