Talanta 197 (2019) 42–48

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Electrochemical aptasensor for ampicillin detection based on the protective effect of aptamer-antibiotic conjugate towards DpnII and Exo III digestion

Tingting Wang[a], Huanshun Yin[b,*], Yuting Zhang[b], Linkui Wang[b], Yue Du[b], Yuping Zhuge[a,*], Shiyun Ai[b]

[a] College of Resources and Environment, Shandong Agricultural University, 271018 Tai'an, Shandong, PR China
[b] College of Chemistry and Material Science, Shandong Agricultural University, 271018 Tai'an, Shandong, PR China

ARTICLE INFO

Keywords:
Antibiotic detection
Ampicillin
Electrochemical biosensor
Exonuclease III
Endonuclease DpnII

ABSTRACT

A simple and sensitive electrochemical method was developed for ampicillin detection based on the protective effect of aptamer-antibiotic conjugate towards endonuclease DpnII activity. Without ampicillin, DNA aptamer firstly hybridizes with the capture probe to form double strand DNA (dsDNA) structure. Then, dsDNA is cleaved by DpnII restriction endonuclease to form two dsDNA fragments. In which, one fragment is released from electrode surface and the other fragment is kept on electrode surface. Then, the dsDNA fragment kept on electrode surface is further digested by Exo III, which leads to the release of the dsDNA fragment from electrode surface. Thus, the electrochemical signal increases due to the decrease of the interface electron transfer resistance causing by the release of dsDNA from electrode surface. However, the formation of dsDNA is blocked when forming aptamer-ampicillin conjugate, which makes the obstruction of the digestion of DpnII and Exo III towards capture probe. Thus, a weak electrochemical signal is achieved due to the increase of the interface electron transfer resistance causing by the dsDNA on the electrode surface. Based on the relationship between ampicillin concentration and the decrease of the electrochemical signal, antibiotic is detected with low detection limit of 32 pM under optimal conditions, which is lower than the mandated maximum residue limit of European Union (9.93 nM). The developed method also presents good detection selectivity. Moreover, the applicability is confirmed by detecting antibiotic in milk and water samples with satisfactory results.

1. Introduction

Ampicillin is a kind of β-lactam antibiotics, which has been widely used to cure various bacterial infections in humans, livestock, poultry, and aquaculture due to the applicability for a wide range of bacterial infections, excellent activity, and tolerability. However, the overuse of antibiotic or antibiotic residues in food and environment can cause serious food safety and environmental safety issues, which is harmful to the health of human and animals. Therefore, it is essential to develop reliable and fast detection method for ampicillin.

To date, various methods have been developed for ampicillin detection, including spectrophotometry [1], high performance liquid chromatography (HPLC) [2], liquid chromatography–mass spectrometry/mass spectrometry (HPLC-MS/MS) [3], fluorescence [4], surface plasmon resonance (SPR) [5], Raman spectroscopy [6], colorimetry [7] and electrochemiluminescence [8]. These methods are sensitive for ampicillin detection, but they require expensive instrument, complicated sample preparation process, and complex instrument operation. Due to the simple operation, time-saving, fast detection, high sensitivity, miniaturized and inexpensive instrument, electrochemical method attracts wide attentions for antibiotic detection [9–13]. Aptamers are artificial single-stranded DNA or RNA nucleic acids, screened by an in vitro selection process termed SELEX (systematic evolution of ligands by exponential enrichment), which can specifically bind with their target molecules with high affinity [14,15]. Compared with antibody, aptamer possesses significant merits, such as low-cost, high reproducibility, small size, good thermostability, easy synthesis and convenient modification. Thus aptamers are a kind of greatly promising reagent to replace antibody. Up to now, aptamers have been widely applied in the field of biosensor for recognizing and detecting various kinds of target molecules, including nucleic acid [16,17], bacteria [18,19], cells [20,21], viruses [22,23], proteins [24,25], phenolic compounds [26,27], pesticides [28,29], antibiotics [30–35], metal ions [36,37], other organic molecules [38,39], etc.

Based on the wide applicability, aptamer-based electrochemical method attracts our attention. Fortunately, the aptamer of ampicillin

* Corresponding authors.
E-mail addresses: yinhs@sdau.edu.cn (H. Yin), zhugeyp@sdau.edu.cn (Y. Zhuge).

https://doi.org/10.1016/j.talanta.2019.01.010
Received 7 November 2018; Received in revised form 25 December 2018; Accepted 2 January 2019
Available online 04 January 2019
0039-9140/

has been synthesized, which has been applied to detect ampicillin with high specificity [40,41]. In this work, a short sequence is added at the 3′-terminal of ampicillin aptamer, which makes that the aptamer sequence containing a recognition site of endonuclease DpnII. It indicates that the double-stranded DNA (dsDNA) containing ampicillin aptamer can be cleaved by DpnII. However, the single-strand probe DNA (ssDNA) can not be digested by DpnII without aptamer DNA. Inspired by it, a simply electrochemical method was developed for ampicillin detection based on protective effect of aptamer-ampicillin conjugate towards the cleavage of dsDNA by DpnII. After aptamer DNA hybridized with probe DNA to form dsDNA structure, this dsDNA can be cleaved by DpnII to form tow fragments of dsDNA (one fragment is released from electrode surface and the other fragment is kept on electrode surface) because there is a recognition region for DpnII in the dsDNA. The DNA fragment kept on electrode surface can be further digested from its 3′-blunt terminus by exonuclease III (Exo III). However, these cleavages can be blocked when aptamer DNA firstly integrated with ampicillin and then released from the electrode surface. The change of the modification of the electrode surface under different conditions can cause the change of the electrochemical signal of the redox probe in detection buffer. Based on it, ampicillin can be detected.

2. Experimental section

2.1. Reagents and instruments

Ampicillin, chloramphenicol, florfenicol, thiamphenicol, tobramycin, streptomycin, tetracycline, kanamycin, oxytetracycline, amoxicillin, penicillin, lincomycin and melamine were provided by Aladdin (Shanghai, China). Exo III and DpnII were provided by New England Biolab (USA). Chloroauric acid ($HAuCl_4$), mercaptopropionic acid (MPA) was supplied by Sigma-Aldrich (USA). Tris(hydroxymethyl) aminomethane (Tris) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were offered by Aladdin (Shanghai, China). DNA was offered by Sangon (Shanghai, China). The sequences were as follows. DNA aptamer, 5′-TTG ATC GCG GGC GGT TGT ATA GCG G-3′, DNA probe, 5′-SH-CCG CAT TAC AAC CGC CCG CGA TCA AAA AA-3′. For DNA aptamer, the italic letter indicates the aptamer sequence and the underline letter indicates the recognition site of DpnII.

The buffers used in this work were as follows. DNA dissolution buffer, 10 mM Tris-HCl and 1 mM EDTA, pH 8.0. Probe immobilization buffer, 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP, pH 7.4. DNA hybridization buffer, 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl. DpnII buffer, 100 mM NaCl , 50 mM Tris-HCl , 10 mM $MgCl_2$, 100 μg/mL BSA (pH 7.9). Exo III buffer, 10 mM Bis Tris propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0. Washing buffer, 10 mM Tris-HCl and 50 mM KCl, pH 7.4. Detection buffer, 10 mM PBS containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl, pH 7.5.

Electrochemical experiments were performed on a CHI660C electrochemical workstation with three-electrode system. Glassy carbon electrode (GCE) or modified GCE was employed as working electrode, saturated calomel electrode was used as reference electrode and platinum wire electrode was used as counter electrode. Differential pulse voltammetry was carried out in 10 mM PBS containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl, pH 7.5. Electrochemical impedance spectroscopy (EIS) was performed in 10 mM PBS containing 5 mM $Fe(CN)_6^{3-/4-}$ (1:1) and 0.1 M KCl, pH 7.5.

2.2. Fabrication of dsDNA/AuNPs/GCE

GCE (d = 3 mm, model CHI104, CH Instruments Ins. USA) was used as substrate electrode. Firstly, GCE was polished to a mirror-like surface with 30 nm Al_2O_3 slurry. Then, the polished GCE was ultrasonically washed with double distilled water and ethanol for 5 min, respectively. After rinsed with double distilled water and dried under N_2 blowing, gold nanoparticles (AuNPs) were modified on GCE surface using potentiostatic deposition technique with the applied potential of −0.2 V for 200 s under magnetic stirring in 10 mL of 3 mM $HAuCl_4$ and 0.1 M KNO_3 solution. The electrode was denoted as AuNPs/GCE. Afterwards, AuNPs/GCE was incubated with 10 μL DNA immobilization buffer containing 1.0 μM DNA probe for 12 h at room temperature under humid condition. After rinsed with washing buffer, the obtained electrode (named as ssDNA/AuNPs/GCE) was blocked with 10 μL of 0.1 mM MPA solution for 60 min at room temperature. Subsequently, ssDNA/AuNPs/GCE was further incubated with 10 μL DNA hybridization buffer containing 1.0 μM DNA aptamer for 120 min at 37 °C in a humid cell. The obtained electrode was marked as dsDNA/AuNPs/GCE.

2.3. Formation of aptamer-ampicillin conjugate

5 μL of DNA hybridization buffer containing 1.0 μM DNA aptamer and 5 μL of different concentrations of ampicillin was mixed together and incubated at room temperature for 60 min (The concentration of aptamer DNA was optimized and the results were listed in Supporting information, Fig. S3). Then, 10 μL of the mixed solution was dripped on ssDNA/AuNPs/GCE surface and incubated for 120 min at 37 °C under humidity condition. After that, the electrode was further blocked with 10 μL of 0.1 mM MPA solution for 60 min at room temperature under humidity condition. The obtained electrode was rinsed with washing buffer and dried under N_2 blowing.

2.4. DpnII and Exo III digestion

For DpnII digestion, 10 μL DpnII reaction buffer containing 100 unit/mL DpnII was dripped on electrode surface and incubated for 80 min at 37 °C in a humid cell. Then, the electrode was rinsed with washing buffer for three times and dried under N_2 blowing.

For Exo III digestion, the electrode surface was immersed with 10 μL Exo III reaction buffer containing 200 unit/mL Exo III for 60 min at 37 °C in a humid cell. After that, the electrode was rinsed with washing buffer for three times and dried under N_2 blowing.

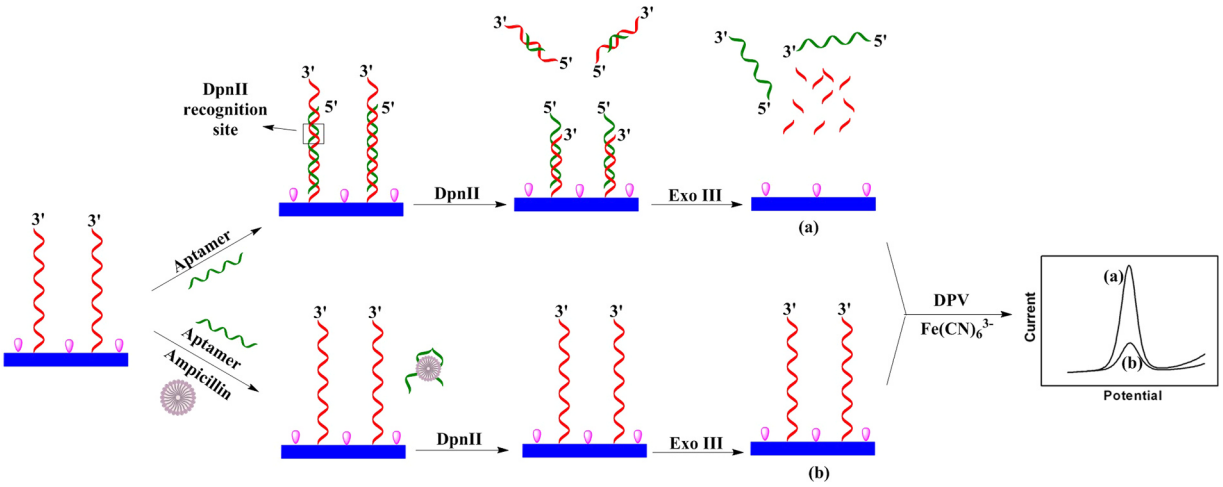
2.5. Detection of ampicillin in milk and water samples

To evaluate the applicability of the developed electrochemical method, ampicillin in milk and water samples was measured. To perform it, different concentrations of ampicillin were spiked into milk and water samples. After centrifugation, 10 μL sample and 10 μL of 1.0 μM DNA aptamer were mixed. Then 10 μL of the mixed solution was dripped onto ssDNA/AuNPs/GCE surface. Afterwards, the electrode was further incubated with DpnII and Exo III successively as described above. After the fabricated electrode was rinsed with washing buffer and dried under N_2 blowing, differential pulse voltammetry was performed in detection buffer with the potential ranging from −0.2–0.6 V.

3. Results and discussion

3.1. Detection strategy

In this work, a simple and sensitive electrochemical strategy was designed for ampicillin detection based on the specific conjugating ability of DNA aptamer to its target of ampicillin, and the specific cleavage ability of DpnII and Exo III. The process for fabricating electrochemical biosensor and detecting ampicillin is shown in Scheme 1. After the pretreatment process of GCE, AuNPs are modified on GCE surface successively by electrochemical deposition. Then, 5′-SH modified DNA probe is further assembled on electrode surface through the formation of Au-S bond. After hybridization, DNA double helix structure (dsDNA) is formed with protruding 3′-terminal with four A bases, which keeps the dsDNA avoiding the digestion of Exo III. However, this dsDNA can be cleavage by DpnII at the palindromic sequence of 5′-¦GATC-3′. As a result, the residual dsDNA fragment with recessed 3′-



Scheme 1. Schematic illustration of electrochemical biosensor for ampicillin detection.

terminal on electrode surface can be further digested by Exo III, which can release the remained DNA fragment from electrode surface. Thus, a strong electrochemical signal can be achieved (Route a). However, as illustrated as route b in Scheme 1, the hybridization of DNA aptamer with DNA probe can be blocked in the presence of ampicillin due to the formation of stable conjugate of aptamer-ampicillin. The immobilized single-strand probe DNA cannot be digested by DpnII and Exo III because the substrate of both them is only dsDNA. Under this condition, the decrease of the electrochemical signal can be achieved. According to the change of the electrochemical signal in the presence and absence of ampicillin, ampicillin concentration can be detected.

3.2. Detection feasibility assay

The electrode modification process is monitored by EIS, and the results are described in Supporting Information (Fig. S1 and S2).

To investigate the detection feasibility of the electrochemical method, several experiments were carried out and the results were discussed. As presented in Fig. 1A, comparing with the bare GCE (curve a, 41.89 μA), AuNPs/GCE (curve b, 200.04 μA) shows a strong electrochemical reduction peak. It can be attributed to the modified AuNPs, which further increase the electrode effective surface area and improve the conductivity of the electrode. However, when DNA probe (curve c), MPA (curve d) and DNA aptamer (curve e) are modified on AuNPs/GCE surface successively, the reduction peak current decrease gradually to 134.76, 93.32, and 73.82 μA, which are caused by the negative charge controlled phosphate skeleton of DNA and MPA, repelling the diffusion of the negative charge controlled redox probe of $Fe(CN)_6^{3-}$. Afterwards, the electrochemical reduction peak current increases quickly when the electrode is incubated with DpnII (curve f, 113.35 μA) and Exo III (curve g, 142.74 μA) successively. This phenomenon demonstrates the successively cleavage of dsDNA by DpnII and Exo III, and the increased current can be ascribed to the reduction of the dsDNA on electrode surface. According to these investigations, the route a can be confirmed.

To further investigate the detection feasibility, several other experiments were performed. As shown in Fig. 1B, curve a is the electrochemical response of dsDNA/AuNPs/GCE (73.82 μA). After ssDNA/AuNPs/GCE is incubated with the mixture of aptamer DNA and 10 nM ampicillin, the electrochemical response increases to 84.35 μA. It can be attributed to the formation of ampicillin-aptamer conjugate, which decreases the hybridization efficiency of DNA probe with DNA aptamer, leads to the reduction of phosphate group amount on electrode surface, and decreases the resistance of the transfer of redox probe. When the electrode is further incubated with DpnII and Exo III, the reduction peak current increases a little to 95.7 (curve c) and 114.1 μA (curve d), respectively. These increases can be ascribed to the digestion of the

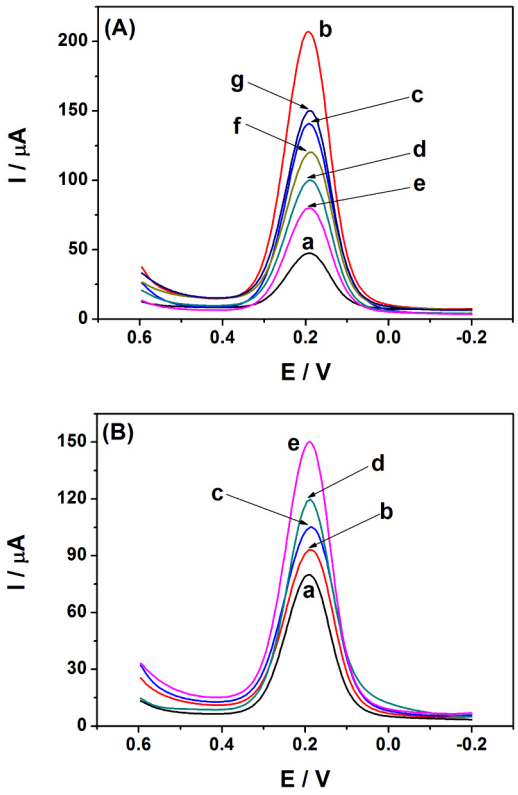


Fig. 1. Differential pulse voltammogram of different electrode in 10 mM PBS (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl. (A) (a) GCE, (b) AuNPs/GCE, (c) ssDNA/AuNPs/GCE, (d) ssDNA/AuNPs/GCE incubated with MPA, (e) dsDNA/AuNPs/GCE, (f) dsDNA/AuNPs/GCE incubated with DpnII, (g) dsDNA/AuNPs/GCE incubated with DpnII and ExoIII, successively. (B) (a) dsDNA/AuNPs/GCE, (b) dsDNA/AuNPs/GCE incubated with 1 nM ampicillin, (c) dsDNA/AuNPs/GCE incubated with ampicillin and DpnII successively, (d) dsDNA/AuNPs/GCE incubated with ampicillin, DpnII and Exo III successively, (e) dsDNA/AuNPs/GCE incubated with DpaII and ExoIII successively.

remained dsDNA on electrode surface by DpnII and Exo III. However, these current values are lower than that obtained at that dsDNA/AuNPs/GCE after directly incubated with DpnII and Exo III successively (curve e, 142.74 μA). Without ampicillin, more dsDNA can be digested by DpnII and Exo III, which significantly decreases the interface electron resistance, and increases the electrochemical response. In the presence of ampicillin, aptamer DNA can be released from electrode

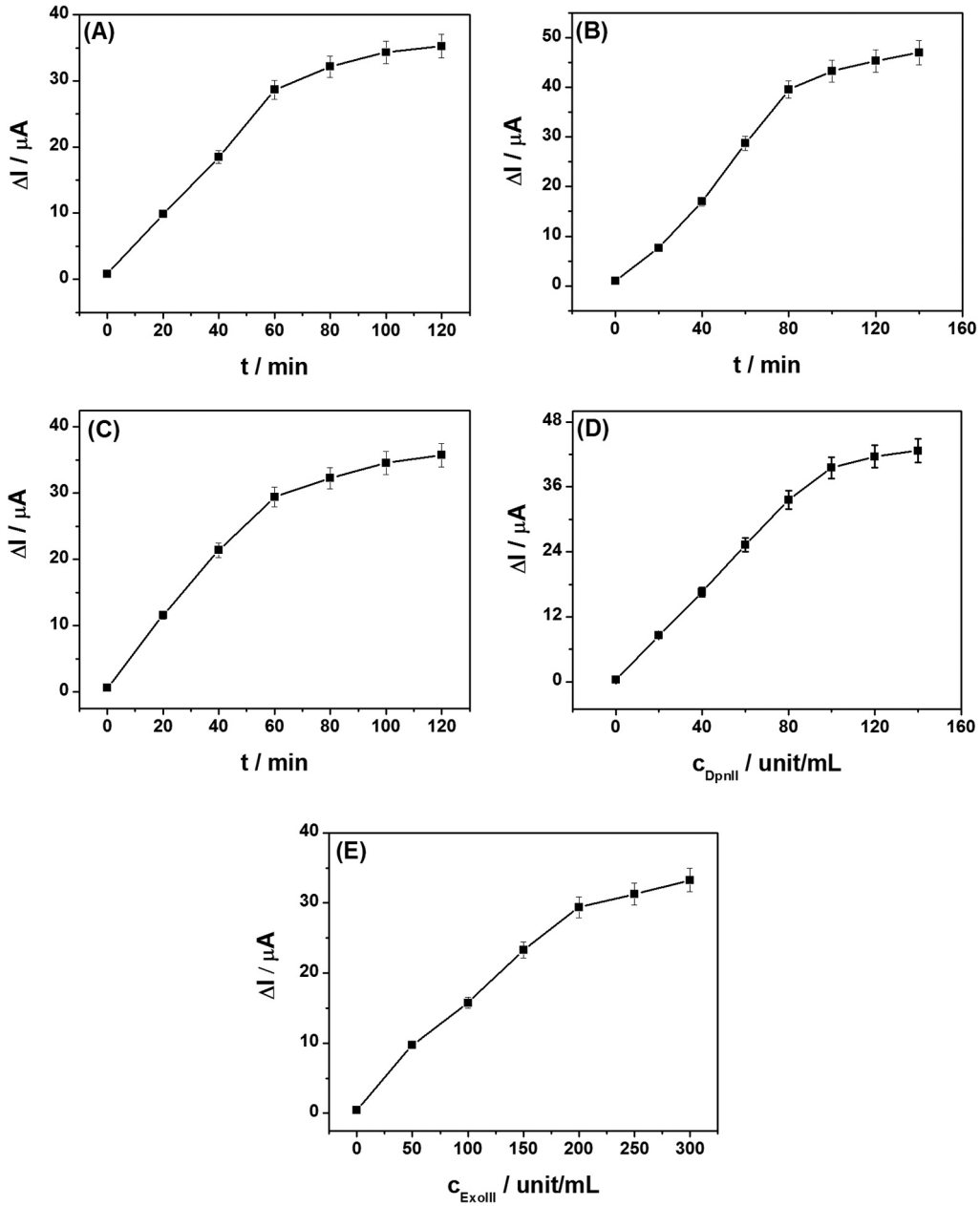


Fig. 2. Effects of binding time for ampicillin and DNA aptamer (A), DpnII (B) and Exo III digestion time (C), DpnII (D) and Exo III (E) concentration on electrochemical signal change.

surface to form aptamer-ampicillin conjugate, which can keep the probe DNA remaining on electrode surface even after the electrode is incubated with DpnII and Exo III. As a result, the electrochemical signal will decrease in the presence of ampicillin. Based on above analysis, the developed method can be applied to detect ampicillin.

3.3. Optimization of experimental conditions

To achieve the high detection sensitivity and high detection efficiency, several experimental parameters were optimized, including binding time of ampicillin with DNA aptamer, DpnII and Exo III digestion time, DpnII and Exo III concentration.

For the optimization of the binding time of ampicillin with DNA aptamer, route b in Scheme 1 is employed. The change of the electrochemical signal ($\Delta I = I_2 - I_1$) is compared, where I_2 is the electrochemical signal of dsDNA/AuNPs/GCE after incubated with DpnII and Exo III successively, and I_1 is the electrochemical signal of ssDNA/AuNPs/GCE after incubated with the mixture of 10 μL of 10 nM ampicillin and 500 nM DNA aptamer, 10 μL of 100 unit/mL DpnII and 10 μL of 200 unit/mL Exo III successively. Fig. 2A shows the effect of binding time for ampicillin and DNA aptamer. With extending binding time from 0 to 60 min, the electrochemical reduction signal decreases gradually. Then a platform is achieved when further increasing binding time. Thus 60 min is chosen as optimal binding time.

To investigate the effect of the incubation time and concentration of DpnII and Exo III on the electrochemical signal, route a in Scheme 1 is selected. The electrochemical signal of dsDNA/AuNPs/GCE after DpnII and Exo III incubation is recorded and the change of the current is compared ($\Delta I = I_2 - I_1$), where I_2 is the electrochemical signal of dsDNA/AuNPs/GCE after incubated with DpnII, or DpnII/Exo III successively, and I_1 is the electrochemical signal of dsDNA/AuNPs/GCE. As shown in Fig. 2B, the electrochemical reduction signal change (ΔI)

increases with increasing DpnII digestion time from 0 to 80 min, then a plateau is obtained when further increasing digestion time, which maybe caused by the approximately saturated digestion of dsDNA by DpnII. Therefore, 80 min is employed. Similarly, Exo III digestion time was also optimized and 60 min is selected as the optimal condition (Fig. 2C).

Fig. 2D illustrates the effect of DpnII concentration on ΔI. With increasing DpnII concentration from 1 to 100 unit/mL, the ΔI increases gradually. When DpnII concentration increases, the digestion amount of dsDNA will also increase, which can decrease the hindrance of electrode surface to the diffusion of the redox probe, and improve the electrochemical reduction signal. As a result, the ΔI increases. However, when further increasing DpnII concentration to 140 unit/mL, the ΔI increases slowly, which might be caused by the approximately saturated digestion of dsDNA. Thus, 100 unit/mL is employed in this work. Fig. 2E presents the optimization of Exo III concentration. According to the optimized results, 200 unit/mL Exo III is selected as the optimal concentration of Exo III.

3.4. Detection performances

To evaluate the detection sensitivity, different concentrations of ampicillin was detected under optimal experimental conditions. As shown in Fig. 3A, the electrochemical reduction peak current decreases with increasing ampicillin concentration. In the range of 0.1–100 nM, the logarithm value of ampicillin concentration is proportional to the reduction peak current (shown in Fig. 3B) and the linear regression equation can be expressed as I_{pc} (μA) = −19.79 log$c$ (nM) +125.82 (R = 0.9937). The detection limit is calculated to be 0.032 nM (3σ), which is lower than the mandated maximum residue limits (MRL) of

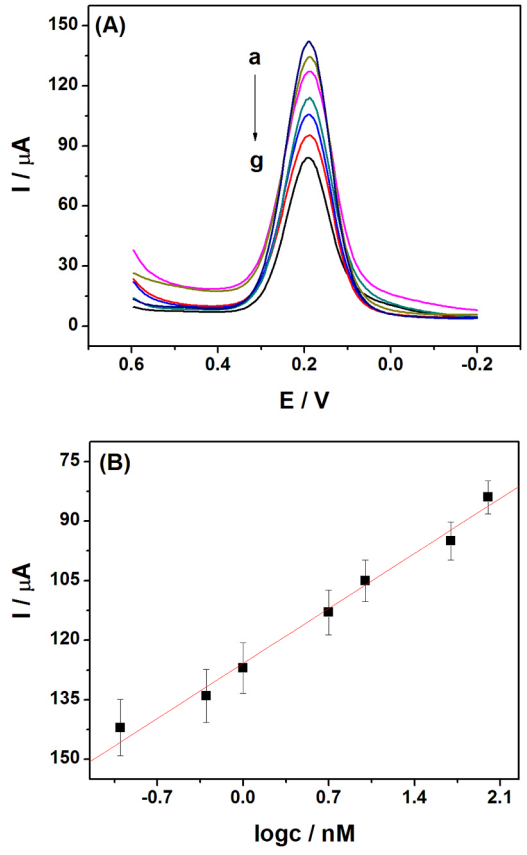


Fig. 3. (A) Differential pulse voltammograms of the biosensor with different concentrations of ampicillin. a-g: 0.1, 0.5, 1, 5, 10, 50, 100 nM. (B) The relationship between the electrochemical signal and the logarithm value of ampicillin concentration.

Table 1
The comparison of the detection performances of the developed method with other reports on ampicillin detection.

Methods	Linear range	Detection limit	Refs.
Electrochemistry	10 pM–100 nM	3 pM	[11]
Electrochemistry	20 pM–40 nM	4 pM	[41]
Electrochemistry	5 pM–10 nM	1.09 pM	[42]
Colorimetry	21–285 nM	14 nM	[43]
Electrochemical microfluidic	100 pM–1 mM	100 pM	[40]
ECL	3 nM–1 μM	1 nM	[8]
HPLC	0.012–1.2 nM	–	[44]
Fluorescence	–	2.48 nM	[45]
Fluorescence	2–10 μM	0.4 μM	[4]
Spectrophotometry	4.96–19.9 μM	–	[46]
Spectrophotometry	4.96–199 μM	3.72 μM	[47]
Electrochemistry	0.1–100 nM	32 pM	This work

European Union (9.93 nM, 4 μg/kg). The detection performances are compared with some of previous work and the results are listed in Table 1.

Detection specificity is an important parameter for assay method, thus it was assessed using chloramphenicol, florfenicol, thiamphenicol, tobramycin, streptomycin, tetracycline, kanamycin, oxytetracycline, penicillin, amoxicillin, lincomycin and melamine as possible interferents. The change of the reduction peak current with different antibiotic ($\Delta I = I_1 - I_2$) was compared, where, I_1 (145.59 μA) is the reduction peak current of dsDNA/AuNPs/GCE after incubated with DpnII, and Exo III successively, and I_2 is the reduction peak current of electrochemical biosensor with 10 nM of different antibiotics. As shown in Fig. 4, ΔI for ampicillin is much higher than that obtained at other antibiotics, indicating that the developed method possesses good detection selectivity.

The detection reproducibility is also a crucial parameter for workable sensor. To evaluate it, seven group of electrochemical biosensors (each group contained seven biosensors) were fabricated independently using 0.1, 1 and 10 nM ampicillin, respectively. The relative standard deviations of the electrochemical signal for these biosensors are 8.36%, 5.23% and 4.28%, respectively. These results imply acceptable detection reproducibility.

3.5. Samples

To investigate the applicability in real samples, the developed electrochemical method was applied to detect ampicillin in milk and water samples. Different concentrations of ampicillin were spiked into these samples. Each sample was performed for six repetitive

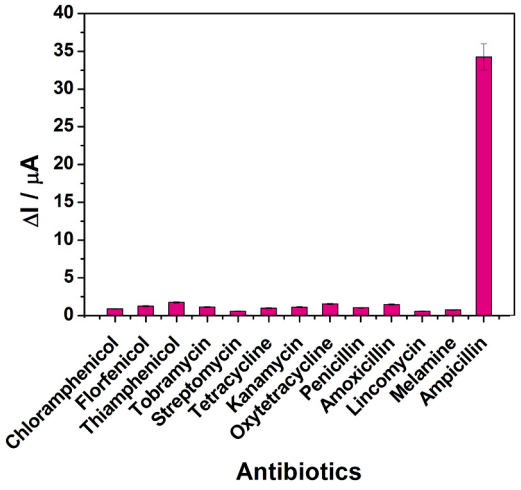


Fig. 4. The currant change of the biosensors with different antibiotics.

Table 2
Detection of ampicillin in milk and water samples.

Samples	Added (μM)	Detected (μM)	Recoveries (%)
Milk 1	0.5	0.48	96
Milk 2	5	4.75	95
Milk 3	10	10.54	105.4
Water 1	0.5	0.47	94
Water 2	5	5.19	103.8
Water 3	10	10.39	103.9

measurements, and acceptable recoveries were obtained in the range from 94% to 105.4% (Table 2). These results indicate that the developed method possesses good anti-interference ability, even in complex samples, which also demonstrate that this electrochemical method has great potential in practical applications.

4. Conclusions

In summary, a simply electrochemical method was constructed for ampicillin detection based on the hindrance of DNA aptamer-ampicillin conjugate towards the digestion of dsDNA by DpnII and Exo III. The use of DNA aptamer can greatly improve the detection selectivity, and the employment of DpnII/Exo III system can effectively increase the detection sensitivity. Thus this electrochemical method shows high detection sensitivity and specificity. Moreover, the applicability of this method was demonstrated using standard addition and recovery experiments with recoveries in the range from 94% to 105.4%.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 21775090, 41771273) and the Natural Science Foundation of Shandong Province of China (No. ZR2016BM10).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2019.01.010.

References

[1] Y.-B. Miao, N. Gan, H.-X. Ren, T. Li, Y. Cao, F. Hu, Y. Chen, Switch-on fluorescence scheme for antibiotics based on a magnetic composite probe with aptamer and hemin/G-quadruplex coimmobilized nano-Pt-luminol as signal tracer, Talanta 147 (2016) 296–301.
[2] M.L. Castillo-Garcí, M.P. Aguilar-Caballos, A. Gómez-Hens, Determination of veterinary penicillin antibiotics by fast high-resolution liquid chromatography and luminescence detection, Talanta 170 (2017) 343–349.
[3] S. Bogialli, V. Capitolino, R. Curini, A. Di Corcia, M. Nazzari, M. Sergi, Simple and rapid liquid chromatography-tandem mass spectrometry confirmatory assay for determining amoxicillin and ampicillin in bovine tissues and milk, J. Agr. Food Chem. 52 (2004) 3286–3291.
[4] A. Fernández-González, R. Badía, M.E. Díaz-García, Micelle-mediated spectrofluorimetric determination of ampicillin based on metal ion-catalysed hydrolysis, Anal. Chim. Acta 484 (2003), pp. 223–231.
[5] E. Gustavsson, J. Degelaen, P. Bjurling, Å. Sternesjö, Determination of β-lactams in milk using a surface plasmon resonance-based biosensor, J. Agr. Food Chem. 52 (2004) 2791–2796.
[6] C. Andreou, R. Mirsafavi, M. Moskovits, C.D. Meinhart, Detection of low concentrations of ampicillin in milk, Analyst 140 (2015) 5003–5005.
[7] Q. Luan, X. Xiong, N. Gan, Y. Cao, T. Li, D. Wu, Y. Dong, F. Hu, A multiple signal amplified colorimetric aptasensor for antibiotics measurement using DNAzyme labeled Fe-MIL-88-Pt as novel peroxidase mimic tags and CSDP target-triggered cycles, Talanta 187 (2018) 27–34.
[8] M.-J. Li, P. Jiao, M. Lin, W. He, G.-N. Chen, X. Chen, High electrochemiluminescence of a new water-soluble iridium(iii) complex for determination of antibiotics, Analyst 136 (2011) 205–210.
[9] A.R. Cardoso, A.P.M. Tavares, M.G.F. Sales, In-situ generated molecularly imprinted material for chloramphenicol electrochemical sensing in waters down to the nanomolar level, Sens. Actuators B Chem. 256 (2018) 420–428.
[10] Y. Zhou, M. Wang, H. Yin, S. Ai, Amperometric determination of the activity of protein kinase a using a glassy carbon electrode modified with IgG functionalized gold nanoparticles conjugated to horseradish peroxidase, Microchim. Acta 184 (2017) 3301–3308.
[11] P. Gai, C. Gu, T. Hou, F. Li, Ultrasensitive self-powered aptasensor based on enzyme biofuel cell and DNA bioconjugate: a facile and powerful tool for antibiotic residue detection, Anal. Chem. 89 (2017) 2163–2169.
[12] S. Huang, N. Gan, T. Li, Y. Zhou, Y. Cao, Y. Dong, Electrochemical aptasensor for multi-antibiotics detection based on endonuclease and exonuclease assisted dual recycling amplification strategy, Talanta 179 (2018) 28–36.
[13] M. Chen, N. Gan, Y. Zhou, T. Li, Q. Xu, Y. Cao, Y. Chen, An electrochemical aptasensor for multiplex antibiotics detection based on metal ions doped nanoscale MOFs as signal tracers and $RecJ_f$ exonuclease-assisted targets recycling amplification, Talanta 161 (2016) 867–874.
[14] A. Alsaafin, M. McKeague, Functional nucleic acids as in vivo metabolite and ion biosensors, Biosens. Bioelectron. 94 (2017) 94–106.
[15] S.D. Jayasena, Aptamers: an emerging class of molecules that rival antibodies in diagnostics, Clin. Chem. 45 (1999) 1628–1650.
[16] M. Wang, Z. Yang, Y. Guo, X. Wang, H. Yin, S. Ai, Visible-light induced photoelectrochemical biosensor for the detection of microRNA based on Bi_2S_3 nanorods and streptavidin on an ITO electrode, Microchim. Acta 182 (2015) 241–248.
[17] Z. Han, M. Luo, L. Chen, H. Pan, J. Chen, C. Li, A photoelectrochemical biosensor for determination of DNA based on flower rod-like zinc oxide heterostructures, Microchim. Acta 184 (2017) 2541–2549.
[18] M. Shahdordizadeh, S.M. Taghdisi, N. Ansari, F. Alebooye Langroodi, K. Abnous, M. Ramezani, Aptamer based biosensors for detection of Staphylococcus aureus, Sens Actuators B Chem. 241 (2017) 619–635.
[19] Y. Guo, Y. Wang, S. Liu, J. Yu, H. Wang, Y. Wang, J. Huang, Label-free and highly sensitive electrochemical detection of *E. coli* based on rolling circle amplifications coupled peroxidase-mimicking DNAzyme amplification, Biosens. Bioelectron. 75 (2016) 315–319.
[20] D. Sun, J. Lu, Y. Zhong, Y. Yu, Y. Wang, B. Zhang, Z. Chen, Sensitive electrochemical aptamer cytosensor for highly specific detection of cancer cells based on the hybrid nanoelectrocatalysts and enzyme for signal amplification, Biosens. Bioelectron. 75 (2016) 301–307.
[21] Y.-S. Borghei, M. Hosseini, M. Dadmehr, S. Hosseinkhani, M.R. Ganjali, R. Sheikhnejad, Visual detection of cancer cells by colorimetric aptasensor based on aggregation of gold nanoparticles induced by DNA hybridization, Anal. Chim. Acta 904 (2016) 92–97.
[22] T.T. Le, B. Adamiak, D.J. Benton, C.J. Johnson, S. Sharma, R. Fenton, J.W. McCauley, M. Iqbal, A.E.G. Cass, Aptamer-based biosensors for the rapid visual detection of flu viruses, Chem. Commun. 50 (2014) 15533–15536.
[23] V.-T. Nguyen, H.B. Seo, B.C. Kim, S.K. Kim, C.-S. Song, M.B. Gu, Highly sensitive sandwich-type SPR based detection of whole H5Nx viruses using a pair of aptamers, Biosens. Bioelectron. 86 (2016) 293–300.
[24] H. Da, H. Liu, Y. Zheng, R. Yuan, Y. Chai, A highly sensitive VEGF165 photoelectrochemical biosensor fabricated by assembly of aptamer bridged DNA networks, Biosens. Bioelectron. 101 (2018) 213–218.
[25] K. Wang, M.-Q. He, F.-H. Zhai, R.-H. He, Y.-L. Yu, A label-free and enzyme-free ratiometric fluorescence biosensor for sensitive detection of carcinoembryonic antigen based on target-aptamer complex recycling amplification, Sens. Actuators B Chem. 253 (2017) 893–899.
[26] Y. Ma, J. Liu, H. Li, Diamond-based electrochemical aptasensor realizing a femtomolar detection limit of bisphenol A, Biosens. Bioelectron. 92 (2017) 21–25.
[27] L. Dai, X. Du, D. Jiang, W. Chen, M. Zhu, K. Wang, Ultrafine α-Fe_2O_3 nanocrystals anchored on N-doped graphene: a nanomaterial with long hole diffusion length and efficient visible light-excited charge separation for use in photoelectrochemical sensing, Microchim. Acta 184 (2017) 137–145.
[28] F. Arduini, S. Cinti, V. Scognamiglio, D. Moscone, Nanomaterials in electrochemical biosensors for pesticide detection: advances and challenges in food analysis, Microchim. Acta 183 (2016) 2063–2083.
[29] R. Bala, M. Kumar, K. Bansal, R.K. Sharma, N. Wangoo, Ultrasensitive aptamer biosensor for malathion detection based on cationic polymer and gold nanoparticles, Biosens. Bioelectron. 85 (2016) 445–449.
[30] Z.-g. Yu, A.L. Sutlief, R.Y. Lai, Towards the development of a sensitive and selective electrochemical aptamer-based ampicillin sensor, Sens. Actuators B Chem. 258 (2018) 722–729.
[31] N. Mohammad Danesh, M. Ramezani, A. Sarreshtehdar Emrani, K. Abnous, S.M. Taghdisi, A novel electrochemical aptasensor based on arch-shape structure of aptamer-complimentary strand conjugate and exonuclease I for sensitive detection of streptomycin, Biosens. Bioelectron. 75 (2016) 123–128.
[32] M. Roushani, K. Ghanbari, S. Jafar Hoseini, Designing an electrochemical aptasensor based on immobilization of the aptamer onto nanocomposite for detection of the streptomycin antibiotic, Microchem. J. 141 (2018) 96–103.
[33] B. Rezaei, H.R. Jamei, A.A. Ensafi, An ultrasensitive and selective electrochemical aptasensor based on rGO-MWCNTs/Chitosan/carbon quantum dot for the detection of lysozyme, Biosens. Bioelectron. 115 (2018) 37–44.
[34] X. Hu, K.Y. Goud, V.S. Kumar, G. Catanante, Z. Li, Z. Zhu, J.L. Marty, Disposable electrochemical aptasensor based on carbon nanotubes-V_2O_5-chitosan nanocomposite for detection of ciprofloxacin, Sensor, Sens. Actuators B Chem. 268 (2018) 278–286.
[35] K. Ghanbari, M. Roushani, A novel electrochemical aptasensor for highly sensitive and quantitative detection of the streptomycin antibiotic, Bioelectrochemistry 120 (2018) 43–48.
[36] S. Zhan, Y. Wu, L. Wang, X. Zhan, P. Zhou, A mini-review on functional nucleic acids-based heavy metal ion detection, Biosens. Bioelectron. 86 (2016) 353–368.
[37] Y. Shi, G. Zhang, J. Li, Y. Zhang, Y. Yu, Q. Wei, Photoelectrochemical determination of Hg(II) via dual signal amplification involving SPR enhancement and a folding-

based DNA probe, Microchim. Acta 184 (2017) 1379–1387.
[38] A.S. Emrani, N.M. Danesh, M. Ramezani, S.M. Taghdisi, K. Abnous, A novel fluorescent aptasensor based on hairpin structure of complementary strand of aptamer and nanoparticles as a signal amplification approach for ultrasensitive detection of cocaine, Biosens. Bioelectron. 79 (2016) 288–293.
[39] N. Dong, Y. Hu, K. Yang, J. Liu, Development of aptamer-modified SERS nanosensor and oligonucleotide chip to quantitatively detect melamine in milk with high sensitivity, Sens. Actuators B Chem. 228 (2016) 85–93.
[40] J. Daprà, L.H. Lauridsen, A.T. Nielsen, N. Rozlosnik, Comparative study on aptamers as recognition elements for antibiotics in a label-free all-polymer biosensor, Biosens. Bioelectron. 43 (2013) 315–320.
[41] X. Wang, S. Dong, P. Gai, R. Duan, F. Li, Highly sensitive homogeneous electrochemical aptasensor for antibiotic residues detection based on dual recycling amplification strategy, Biosens. Bioelectron. 82 (2016) 49–54.
[42] H. Wang, Y. Wang, S. Liu, J. Yu, W. Xu, Y. Guo, J. Huang, Target-aptamer binding triggered quadratic recycling amplification for highly specific and ultrasensitive detection of antibiotics at the attomole level, Chem. Commun. 51 (2015) 8377–8380.
[43] K.-M. Song, E. Jeong, W. Jeon, M. Cho, C. Ban, Aptasensor for ampicillin using gold nanoparticle based dual fluorescence-colorimetric methods, Anal. Bioanal. Chem. 402 (2012) 2153–2161.
[44] B.C. McWhinney, S.C. Wallis, T. Hillister, J.A. Roberts, J. Lipman, J.P.J. Ungerer, Analysis of 12 beta-lactam antibiotics in human plasma by HPLC with ultraviolet detection, J. Chromatogr. B 878 (2012) 2039–2043.
[45] M.A. Bacigalupo, G. Meroni, F. Secundo, R. Lelli, Time-resolved fluoroimmunoassay for quantitative determination of ampicillin in cow milk samples with different fat contents, Talanta 77 (2008) 126–130.
[46] E.G. Totoli, H.R. Nunes Salgado, Development and validation of a rapid turbidimetric assay to determine the potency of ampicillin sodium in powder for injectable solution, Anal. Methods 5 (2013) 5923–5928.
[47] L. Xu, H. Wang, Y. Xiao, Spectrophotometric determination of ampicillin sodium in pharmaceutical products using sodium 1,2-naphthoquinone-4-sulfonic as the chromogentic reagent, Spectrochim. Acta A Mol. Biomol. Spectrosc. 60 (2004) 3007–3012.