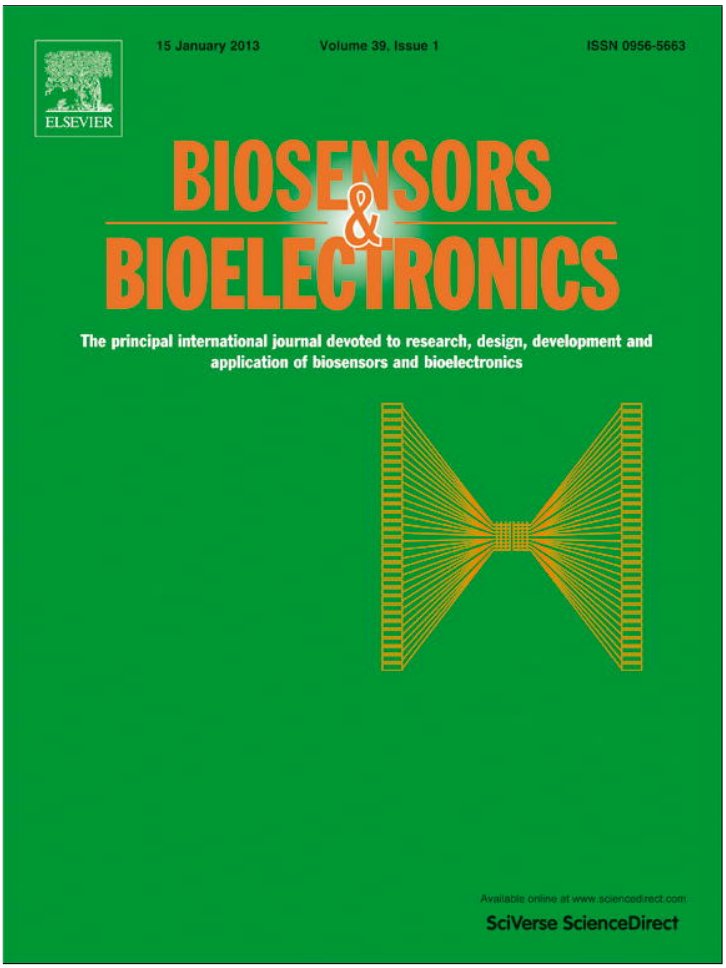


Biosensors and Bioelectronics 39 (2013) 199–203

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Sub-femtomolar electrochemical detection of DNA using surface circular strand-replacement polymerization and gold nanoparticle catalyzed silver deposition for signal amplification

Fenglei Gao, Zhu Zhu, Jianping Lei, Yao Geng, Huangxian Ju *

State Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, PR China

ARTICLE INFO

Article history:
Received 5 June 2012
Received in revised form
19 July 2012
Accepted 20 July 2012
Available online 27 July 2012

Keywords:
Electrochemical biosensors
Circular strand-displacement polymerization
Signal amplification
Silver deposition
DNA

ABSTRACT

A highly sensitive method was developed for detection of target DNA. This method combined circular strand-displacement polymerization (CSRP) with silver enhancement to achieve dual signal amplification. After molecular beacon (MB) hybridized with target DNA, the reporter gold nanoparticle (Au NPs) was attached to an electrode surface by hybridization between Au NP labeled primer and stem part of the MB to initiate a polymerization of DNA strand, which led to the release of target and another polymerization cycle. Thus the CSRP produced the multiplication of target-related reporter Au NPs on the surface. The Au NPs then catalyzed silver deposition for subsequent stripping analysis of silver. The dual signal amplification offered a dramatic enhancement of the stripping response. This signal could discriminate perfect matched target DNA from 1-base mismatch DNA. The dynamic range of the sequence-specific DNA detection was from 10^{-16} to 10^{-12} mol L^{-1} with a detection limit down to sub-femtomolar level. This proposed method exhibited an efficient amplification performance, and would open new opportunities for sensitive detection of other biorecognition events.

1. Introduction

The ultrasensitive detection of DNA hybridization has attracted considerable interest in different fields such as clinical diagnosis, food safety and environmental protection (Mao et al., 2009; Xia et al., 2010; Huang et al., 2011; Wang et al., 2011). Many signal amplification strategies based on the loading of a large number of signal molecules on nanocarrier to label the recognition molecule have been designed to improve the sensitivity in the detection of DNA. Several methods using enzyme as the signal molecules have been developed for the detection of femtomolar DNA targets (Patolsky et al., 1999; Azek et al., 2000; Hwang et al., 2005; Liu et al., 2008). However, the easy denaturation of enzyme in wild environment blocks their applications in practice. As substitutes, nanoparticles (NPs), such as metal NPs, metal oxide NPs and semiconductor quantum dots, have been extensively used as signal molecules to significantly enhance the sensitivity and stability due to the unique properties of NPs (Zhang et al., 2009; Gao et al., 2011). These NPs assembled on nanocarriers as the signal tags have been combined with different analytical techniques for the development of genosensing fields (Wang, 2003).

DNA amplification is another interesting alternative for highly sensitive detection of DNA. Besides the polymerase chain reaction (Ye et al., 2003), rolling circle amplification (RCA) (Cheng et al., 2010), hybridization chain reaction (HCR) (Huang et al., 2011), loop-mediated amplification (Hsieh et al., 2012) and target DNA recycling amplification with endonuclease (Chen et al., 2011) or exonuclease (Zuo et al., 2010) have also been used for the development of highly sensitive biosensing methods. The approaches based on endonuclease have been demonstrated to be able to detect DNA down to 10^{-14} M (Kiesling et al., 2007; Li et al., 2008; Xu et al., 2009; Chen et al., 2010). Unfortunately, endonuclease is sequence-specific, which limits its application, although a method based on Y-shaped junction DNA has been proposed (Wang et al., 2012). Thus polymerase-based circular strand-replacement polymerization (CSRP) has recently attracted considerable attention in target recycling amplification (Guo et al., 2009). This technique uses lengthening of a new strand to replace the target sequence, and thus releases the target to initiate a new polymerization cycle. CSRP does not require a specific recognition site, thus can be conveniently used for designing DNA sensors. For example, our previous work performed the CSRP in solution to develop a fluorescence quenching-based method for detection of microRNA (Dong et al., 2012). This work combined the CSRP technique with NPs-based signal amplification to design a novel dual signal amplification strategy for electrochemical detection of target DNA. The NPs-based signal

* Corresponding author. Tel./fax: +86 25 83593593.
E-mail address: hxju@nju.edu.cn (H. Ju).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.07.035

amplification was performed using a gold NP catalyzed silver deposition process (Scheme 1).

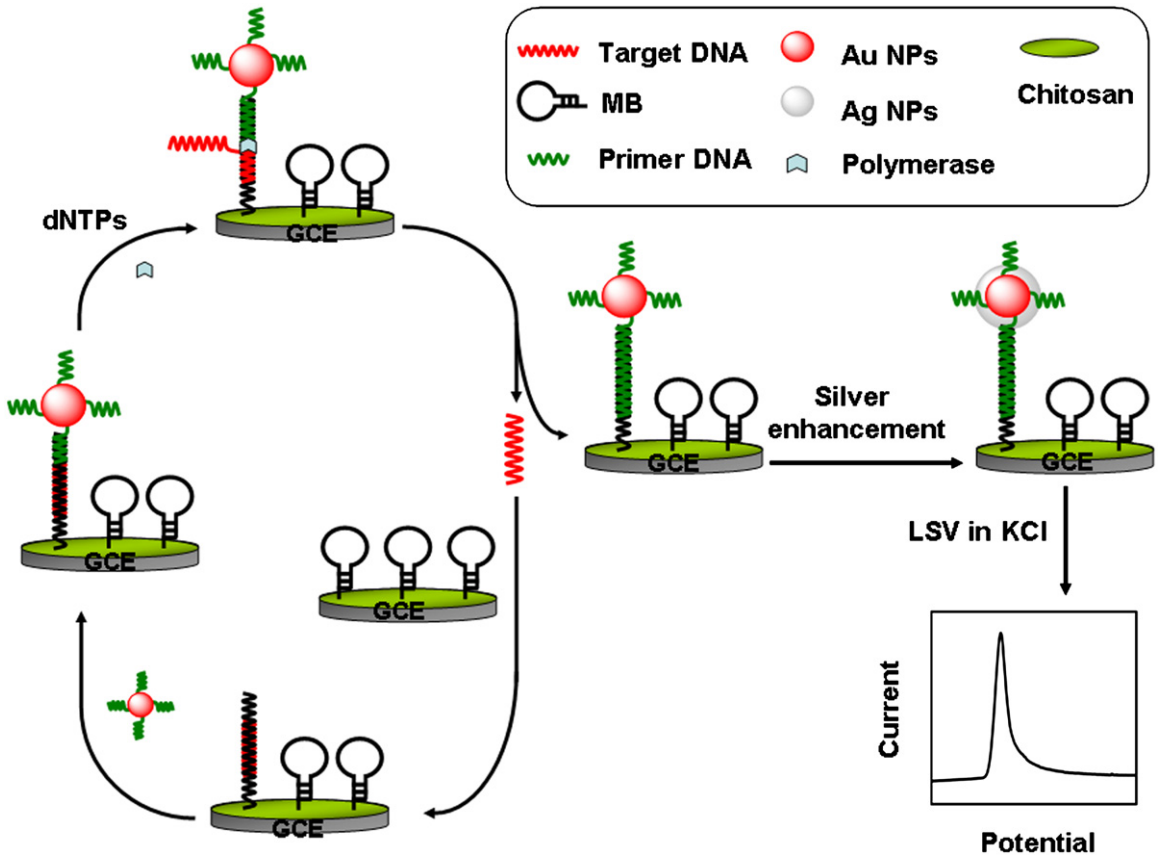
Silver deposition on Au NPs is a common way to provide a visual signal for probe-conjugated Au NPs, which can be read out by optical scanometry (Storhoff et al., 1998; Taton et al., 2000), electrochemical stripping analysis of deposited Ag NPs (Lai et al., 2011) and even surface-enhanced Raman scattering (Lim et al., 2010). This technique can lead to two to three orders of magnitude improvement in detection sensitivity (Taton et al., 2000). In addition, Ag NPs can be oxidized at more negative potential to produce a relatively sharper peak than Au NPs (Lai et al., 2011), which is favorable for obviating the interference of reducing species and improving the analytical performance. Here, a chitosan modified glassy carbon was used to bind molecular beacon (MB) for DNA sensor preparation. More importantly, the CSRP process was also integrated on the surface using primer-loaded Au NPs, which led to the in situ amplification of both CSRP product and Ag NPs. Thus the electrochemical detection of DNA hybridization could be conveniently performed. The biosensing system included two parts: target recycling amplification of CSRP and electrochemical stripping analysis of silver as signal label. As shown in Scheme 1, the target sequence solution containing deoxyribonucleotides (dNTPs), polymerase and primer-load Au NPs was first dropped onto the sensor surface to hybridize with MB and opened the loop part. The opened stem then hybridized with the primer assembled on Au NPs to initiate polymerization of DNA strand, which led to the release of the target. The released target found another MB to trigger the polymerization cycle, resulting in the multiplication of the reporter Au NPs on the sensor surface. Sequentially, the Au NPs-promoted silver deposition afforded a signal trace for electrochemical stripping analysis of target DNA. This method could avoid de-oxygenation procedure for usual electrochemical detection, thus had a promising application in clinical diagnosis.

2. Materials and methods

2.1. Materials and reagents

DNA polymerase and the mixture of deoxyribonucleotides (dNTPs) were obtained from Fermentas Biotechnology Co. Ltd. (Canada). Chitosan (CS, M.W. 100,000–300,000, deacetylation degree $\geq$95%), glutaraldehyde (GA, 25%), albumin from bovine serum (BSA), polyethylene glycol sorbitan monolaurate (TWEEN-20), dithiolthreitol (DTT), tris (2-carboxyethyl) phosphane hydrochloride (TCEP), tris (hydroxymethyl) aminomethane (Tris), and silver-enhancer kit including enhancement solutions A and B were purchased from Sigma-Aldrich (USA). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were obtained from Shanghai Reagent Co. (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (18 MΩ, Milli-Q, Millipore) was used in all assays. DNA hybridization buffer was phosphate-buffered saline (137 mM NaCl, 2.5 mM Mg^{2+}, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4, pH 7.4). DNA was stored in Tris-HCl (10 mM, pH 8.0) containing 1 mM ethylenediaminetetraacetic acid. The polymerase buffer contained 100 nM PBS (pH 7.5), 15 mM $MgCl_2$, and 1 mM DTT. Phosphate-buffered saline (PBS, 0.1 M) of various pH was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. The washing buffer was PBS (0.1 M, pH 7.4) containing 0.05% (w/v) Tween-20.

The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified



Scheme 1. Schematic illustration of dual signal amplification strategy for DNA detection by CSRP and Au NPs catalyzed silver enhancement.

using high-performance liquid chromatography. Their sequences were as follows:

target: 5′- **GAA CAG CCA CCG AAC**-3′
NH_2-modified molecular beacon (MB): NH_2-ATC GAT TAC CGC **GTT CGG TGG CTG TTC** TAC GTA ATC GAT-3′
SH-modified primer: SH-AAA AAA AAA ATC GAT TAC-3′
single-base mismatched: 5′-**GAA CAG CTA CCG AAC**-3′
non-complementary: 5′-**TGC ATC GGC AAC CCA**-3′

2.2. Apparatus

Linear sweep stripping voltammetric (LSV) measurements were performed using a CHI 660B electrochemical workstation (CHI, Shanghai, China) at room temperature using a conventional three-electrode system with a modified glassy carbon electrode (GCE) as the working electrode, a platinum wire as the auxiliary, and a saturated calomel electrode as the reference. Electrochemical impedance spectroscopic (EIS) analysis was performed with an Autolab PGSTAT12 (Ecochemie) in 0.1 M KCl containing 5 mM $[Fe(CN)_6]/K_3[Fe(CN)_6]$.

2.3. Preparation of primer-Au NPs

First, the colloidal Au NPs with 13-nm diameter were prepared according to the previous protocol.[S1] Briefly, 100 mL of 0.01% $HAuCl_4$ solution was boiled with vigorous stirring, and 2.5 mL of 1% trisodium citrate solution was quickly added to the boiling solution. The solution turned deep red, indicating the formation of Au NPs. Followed by continued stirring and cooling down, the resulting Au colloidal solution was stored in brown glass bottles at 4 °C before use. Before DNA loading, the thiol functionality on the oligonucleotide probes was deprotected by treatment with 1.7 equivalents of TCEP for 1 h by using acetate buffer (0.05 mM, pH 5.2) at room temperature. The Au NPs (3 mL, 2.5 nM) were functionalized with the deprotected thiololigonucleotides by incubation at room temperature for at least 16 h with gently stirring and an additional 24 h after the concentration of NaCl had been increased to 100 mM. Afterward, 0.3 mL of blocking buffer (10 mM PBS containing 0.1 M NaCl, 0.02% Tween-20 and 10% BSA, pH 7.4) was dropped into the solution. After stirring for 2 h, the excess of DNA, and BSA were removed by centrifugation (12,000 rpm, 30 min at 4 °C) and redispersed in 10 mM PBS containing 0.1 M NaCl, which was repeated for further purification. The obtained primer-Au NPs were redispersed in 2 mL of 10 mM pH 7.4 PBS containing 0.1 M NaCl and stored at 4 °C.

2.4. Immobilization of MB

Before modification, a GCE with 3-mm diameter was polished to a mirror using 1.0, 0.3, and 0.05 μm alumina slurry followed by rinsing thoroughly with deionized water, and allowed to dry at room temperature. Then, 5 μL of 0.25 mg/mL CS was dropped on the surface of the cleaned electrode, and dried at room temperature. After activating with 2.5% GA (in 50 mM, pH 7.4 phosphate buffer) for 2 h and washing with water, 5 μL MB (1 μM) was dropped on the GA modified electrode for another 2 h to covalently immobilize the 5-NH_2 modified MB, and thus the DNA biosensor was obtained after washing with 10 mM PBS buffer solution.

2.5. Strand-replacement DNA polymerization

50 μL duplication solution containing the target DNA with the designed concentration, 5 μL of primer-Au NPs (0.394 nM), 4 U polymerase, and dNTPs (12 μM for each component) in polymerase buffer were dropped on the surface of the electrodes. After the duplication process was performed for a certain period at 37 °C, it was terminated by washing thoroughly. The whole procedure was shown in Scheme 1.

2.6. Measurement procedure

5 μL of silver deposition solution containing a 1:1 mixture of the silver enhancement solutions A and B was delivered to the electrode for 4 min. After silver deposition, the electrode was rinsed with water and LSV from −0.15 to 0.25 V at 50 mV s^{-1} was performed in a 1.0 M KCl solution.

2.7. Gel electrophoresis

A 20% polyacrylamide gel electrophoresis analysis of the products via the isothermal strand-displacement polymerization reaction was carried out in 1 × Tris-borate-EDTA (pH 8.3) at 100 V constant voltage for about 2 h. After ethidium bromide staining, gels were scanned using a Molecular Imager Gel Doc XR (BIO-RAD, USA).

3. Results and discussion

3.1. Verification of CSRP product

First of all, it is essential to confirm the polymerization as expected for amplification of DNA detection signal. As shown in Fig. 1, in the presence of dNTPs and polymerase, the recognition of MB modified electrode to target 10 fmol L^{-1} DNA led to an obvious stripping peak (curve a), which was 4.2 times greater than that in the absence of dNTPs and polymerase (curve b), showing obvious signal amplification. In the absence of target, the stripping peak for the mixture of polymerase and dNTPs was also very low (curve c), which was the same as the blank (curve d), indicating that no polymerization reaction was triggered. Thus the DNA target could hybridize with the immobilized MB to open the cycle of MB and the opened stem part of MB could then bind

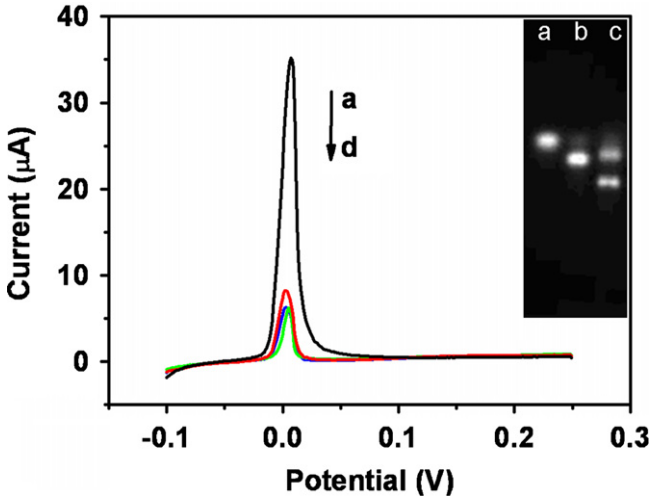


Fig. 1. Linear sweep stripping voltammetric curves of Ag NPs deposited at sensor surface (50 mV s^{-1} in 1.0 M KCl) in the presence of 10 fmol L^{-1} target, dNTPs and polymerase (a), 10 fmol L^{-1} target (b), dNTPs and polymerase (c), and blank (d). Inset: PAGE analysis of the products via the isothermal CSRP with 1.0×10^{-7} M MB (a), 1.0×10^{-7} M MB, 0.5×10^{-7} M primer and target (b), 1×10^{-7} M MB, 0.5×10^{-7} M primer and target in the presence of polymerase and dNTPs (c).

the primer assembled Au NPs to the surface of electrode to initiate the polymerization of DNA strand, which led to the release of target DNA for rehybridization with another immobilized MB. The CSRP amplified the attachment of Au NPs on the sensor surface and greatly improved the sensitivity of detection.

The polymerization reaction was further confirmed with a 20% polyacrylamide gel electrophoresis (PAGE) analysis (Fig. 1, inset). MB exhibited only one band (lane a). After target and primer were added to the solution, a new band appeared (lane b), which corresponded to the formed double stranded DNA. After polymerase and dNTPs were further added to the mixture, a new product band with a slow migration speed was observed (lane c). This result should be contributed to an intermediate product of MB-target-polymerization DNA complex existing in the process of primer extension before the target was displaced, verifying the polymerization process.

The surface polymerization process could be identified with electrochemical impedance spectroscopy (EIS) (Fig. 2). When the bare GCE was coated with a layer of chitosan and then bound to MB, the charge-transferring resistance (R_{ct}) increased gradually (curves a–c). R_{ct} further increased after the target DNA was hybridized with MB, since an opened negatively charged interface electrostatically repelled the negatively charged $[Fe(CN)_6]^{3-/4-}$ redox probe and inhibited interfacial charge transfer (curve d). However, the R_{ct} slightly decreased when the primer assembled Au NPs were attached to the opened stem part due to the excellent electronic conductivity of Au NPs (curve e). R_{ct} further decreased after CSRP (curve f), indicating more Au NPs were attached on the surface with the help of CSRP. This is consistent with the increased stripping current of Ag NPs after CSRP (Fig. 1, curve a).

3.2. Optimization of detection conditions

The CSRP process could be affected by a series of factors including the amount of polymerase and the duplication time. The peak current of stripping response was plotted against the amount of the polymerase in the range from 0 to 5 U (Fig. S1A). The signal increased with the increasing amount of polymerase until the enzyme amount exceeded 4 units, which was then chosen as advised. The time duration of the duplication process was examined from 0 to 3.0 h (Fig. S1B). After 2.5 h the signal reached a plateau, so 2.5 h was chosen as the duplication time.

In order to obtain high sensitivity, the ratio of signal to noise was used to optimize the silver deposition conditions. Upon the successive dilution of silver enhancer solution, the signal-to-noise ratio quickly increased and trended to a maximum value at 20-fold dilution (Fig. S1C). Moreover, since high concentration of silver enhancer solution could produce high background current, the enhancer solution with 20-fold dilution was used for silver deposition, at which the signal-to-noise ratio could be kept at a high level within the deposition time of 4 min (Fig. S1D). Considering the high detection signal obtained at longer deposition time, 4 min was selected as the optimal deposition time.

3.3. Sensitivity for target detection

Under optimal conditions, the DNA assay was performed with a series of target DNA concentrations. As shown in Fig. 3, the peak current was logarithmically proportional to the target concentration in the range from 100 aM to 1 pM. The detection limit was 0.03 fmol L^{-1} at 3 times the standard deviation of the control (free of target DNA). A comparison between the present biosensor and previously reported assays is listed in Table 1. The proposed biosensor showed a much lower limit of detection. The detection limit of 0.03 fmol L^{-1} corresponds to the detection of 0.15 zmol of target in a 5 μL of sample solution. In order to confirm that the high sensitivity of DNA detection resulted from the CSRP and following silver deposition, control experiments with target at various concentrations from 1.0×10^{-12} to 1.0×10^{-8} mol L^{-1} were carried out in the absence of polymerase (Fig. S2). The stripping peak current increased with the increasing target concentration with a detection limit of only 2.88 fmol, which was 100 times higher than that obtained in the presence of polymerization.

3.4. Selectivity of target detection

The selectivity of the silver enhancement and CSRP-based amplification was studied using three kinds of DNA sequence including perfectly complementary targets, one-base mismatched strands and non-complementary strands, at a concentration of 10 fmol L^{-1} (Fig. 4). The perfectly complementary target showed a response 4.4 times that of the single-base mismatch sequence, indicating good selectivity. This high specificity arose from the conformational constraint of the stem-loop structure of MB (Bonnet et al., 1999); that is, the presence of the stem made it

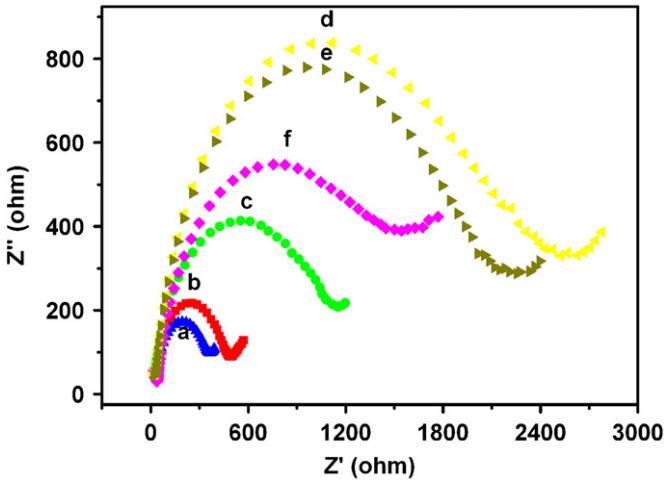


Fig. 2. EIS in 0.1 M KNO_3 containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ at (a) bare GCE, (b) CS modified GCE, (c) (b)+immobilization of MB, (d) (c)+hybridization with target sequence, (e) (d)+attaching of primer-Au NPs; (f) (e) after duplication for 2 h.

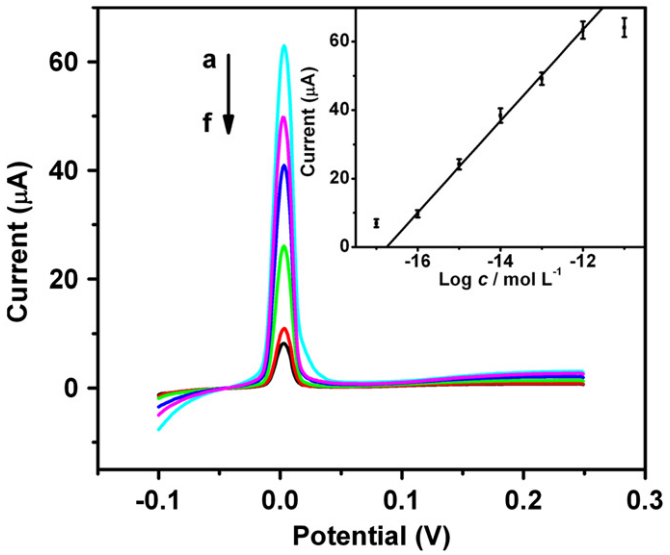


Fig. 3. Linear sweep stripping voltammetric curves of Ag NPs for detection of DNA after duplication at target DNA concentrations of 10^{-17}–10^{-12} mol L^{-1} (curves (a)–(f). Inset: linear relationship between peak current and the logarithm of target DNA concentration.

Table 1
Comparison between the proposed assay and other reported method for DNA detection.

Method	Analytical technique	Linear range (mol L^{-1})	Detection limit (mol L^{-1})	References
HCR	Fluorescence	5×10^{-7}–2×10^{-13}	10^{-15}	Huang et al. (2011)
RCA	Raman	10^{-7}–10^{-11}	10^{-11}	Hu and Zhang (2010)
Amplification by exonuclease	Electrochemical impedance	10^{-8}–10^{-10}	4.2×10^{-11}	Xu et al. (2012)
CSRP	Fluorescence	10^{-10}–6.4×10^{-15}	6.4×10^{-15}	Guo et al. (2009)
CSRP	LSV	10^{-12}–10^{-16}	3×10^{-17}	Present work

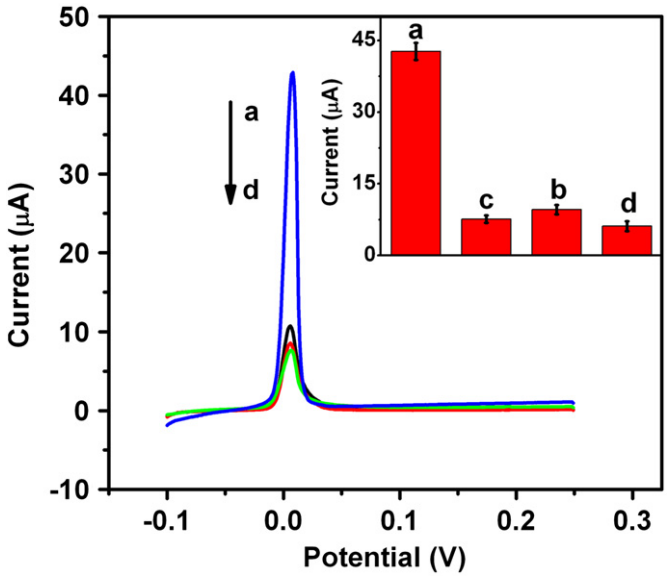


Fig. 4. Linear sweep stripping voltammetric curves of Ag NPs for detection of DNA (a) complementary, (b) single-base mismatch, (c) non-complementary sequences, and (d) blank. Inset: histograms for current values.

thermodynamically unfavorable for the binding of the mismatched sequence to the loop. The response of the non-complementary strand is only 16% that of the perfectly complementary target, which mainly resulted from the background. These results demonstrated that the electrochemical DNA biosensor was able to detect effectively a target with high specificity, and had great potential for single nucleotide polymorphism analysis.

4. Conclusions

This work proposed a novel electrochemical method for detection of DNA hybridization by combining polymerase-induced CSRP with Au NPs catalyzed silver deposition on the surface of MB-based sensor. The dual amplification led to an extremely high sensitivity with a sub-femtomolar detection limit. The detectable concentration was in a linear range of 4 orders of magnitude. The excellent selectivity to differentiate single-base mismatched sequences of DNA was verified due to the intrinsic functions of MB and polymerase. The electrochemical oxidation of Ag NPs in KCl excluded specific detection conditions, such as pretreatment of the NPs, high stripping potential, and de-oxygenation procedure. Therefore, the method presented here provided a universal platform to detect DNA at an ultrasensitive level in biomedical and bioanalytical applications.

Acknowledgments

We gratefully acknowledge the National Basic Research Program of China (2010CB732400), the National Science Fund for Creative Research Groups (21121091) and the project (21135002) from National Natural Science Foundation of China.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.07.035.

References

Azek, F., Grossiord, C., Joannes, M., Limoges, B., Brossier, P., 2000. Analytical Biochemistry 284, 107–113.
Bonnet, G., Tyagi, S., Libchaber, A., Kramer, F.R., 1999. Proceedings of the National Academy of Sciences 96, 6171–6176.
Chen, J.H., Zhang, J., Li, J., Fu, F.F., Yang, H.H., Chen, G.N., 2010. Chemical Communications 46, 5939–5941.
Chen, J.H., Zhang, J., Guo, Y., Li, J., Fu, F.F., Yang, H.H., Chen, G.N., 2011. Chemical Communications 47, 8004–8006.
Cheng, W., Yan, F., Ding, L., Ju, H.X., Ying, Y.B., 2010. Analytical Chemistry 82, 3337–3342.
Dong, H.F., Zhang, J., Ju, H.X., Lu, H.T., Wang, S.Y., Jin, S., Hao, K.H., Du, H.W., Zhang, X.J., 2012. Analytical Chemistry 84, 4587–4593.
Gao, W.C., Dong, H.F., Lei, J.P., Ji, H.X., Ju, H.X., 2011. Chemical Communications 47, 5220–5222.
Guo, Q.P., Yang, X.H., Wang, K.M., Tan, W.H., Li, W., Tang, H.X., Li, H.M., 2009. Nucleic Acids Research 37, e20.
Hwang, S., Kim, E., Kwak, J., 2005. Analytical Chemistry 77, 579–584.
Hsieh, K., Patterson, A.S., Ferguson, B.S., Plaxco, K.W., Soh, H.T., 2012. Angewandte Chemie International Edition 132, 10.1002/anie, 201109115.
Huang, J., Wu, Y.R., Chen, Y., Zhu, Z., Yang, X.H., Yang, C.Y.J., Wang, K.M., Tan, W.H., 2011. Angewandte Chemie International Edition 50, 401–404.
Hu, J., Zhang, C.Y., 2010. Analytical Chemistry 82, 8991–8997.
Kiesling, T., Cox, K., Davidson, Dretchen, E.A., K., Grater, G., Hibbard, S., Lasken, R.S., Leshin, J., Skowronski, E., Danielsen, M., 2007. Nucleic Acids Research 35, e117.
Lai, G.S., Yan, F., Wu, J., Leng, C., Ju, H.X., 2011. Analytical Chemistry 83, 2726–2732.
Li, J.J., Chu, Y., Lee, B.Y.H., Xie, X.S., 2008. Nucleic Acids Research 36, e36.
Lim, D.K., Jeon, K.S., Kim, H.M., Nam, J.M., Suh, Y.D., 2010. Nature Materials 9, 60–67.
Liu, G., Wan, Y., Gau, V., Zhang, J., Wang, L.H., Song, S.P., Fan, C.H., 2008. Journal of the American Chemical Society 130, 6820–6825.
Mao, X., Xu, H., Zeng, Q.X., Zeng, L.W., Liu, G.D., 2009. Chemical Communications 45, 3065–3067.
Patolsky, F., Katz, E., Bardea, A., Willner, I., 1999. Langmuir 15, 3703–3706.
Storhoff, J.J., Elghanian, R., Mucic, R.C., Mirkin, C.A., Letsinger, R.L., 1998. Journal of the American Chemical Society 120, 1959–1964.
Taton, T.A., Mirkin, C.A., Letsinger, R.L., 2000. Science 289, 1757–1760.
Wang, F., Elbaz, J., Orbach, R., Magen, N., Willner, I., 2011. Journal of the American Chemical Society 133, 17149–17151.
Wang, J., 2003. Analytica Chimica Acta 500, 247–257.
Wang, Q., Yang, L.J., Yang, X.H., Wang, K.M., He, L.L., Zhu, J.Q., Su, T.Y., 2012. Chemical Communications 48, 2982–2984.
Xia, F., White, R.J., Zuo, X.L., Patterson, A., Xiao, Y., Kang, D., Gong, X., Plaxco, K.W., Heege, A.J., 2010. Journal of the American Chemical Society 132, 14346–14348.
Xu, H.F., Wang, L.L., Ye, H.Z., Yu, L.S., Zhu, X., Lin, Z.Y., Wu, G.W., Li, X.H., Liu, X.X., Chen, G.N., 2012. Chemical Communication 48, 6390–6392.
Xu, W., Xue, X.J., Li, T.H., Zeng, H.Q., Liu, X.G., 2009. Angewandte Chemie International Edition 48, 6849–6852.
Ye, Y.K., Zhao, J.H., Yan, F., Zhu, Y.L., Ju, H.X., 2003. Biosensors and Bioelectronics 18, 1501–1508.
Zhang, J., Ting, B.P., Jana, N.R., Gao, Z.Q., Ying, J.Y., 2009. Small 5, 1414–1417.
Zuo, X.L., Xia, F., Xiao, Y., Plaxco, K.W., 2010. Journal of the American Chemical Society 132, 1816–1818.