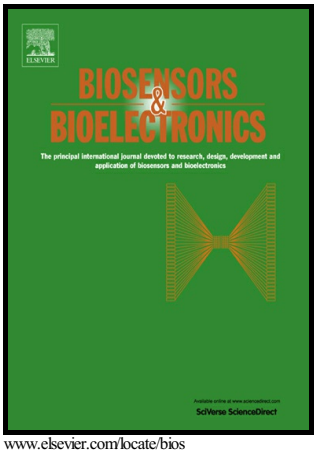


www.elsevier.com/locate/bios

Label-free Electrochemical Nucleic Acid Biosensing by Tandem Polymerization and Cleavage-Mediated Cascade Target Recycling and DNAzyme Amplification

Shufeng Liu, Hongwei Gong, Yanqun Wang, Li Wang

PII: S0956-5663(15)30518-2
DOI: http://dx.doi.org/10.1016/j.bios.2015.10.056
Reference: BIOS8092

To appear in: *Biosensors and Bioelectronic*

Received date: 9 September 2015
Revised date: 18 October 2015
Accepted date: 19 October 2015

Cite this article as: Shufeng Liu, Hongwei Gong, Yanqun Wang and Li Wang, Label-free Electrochemical Nucleic Acid Biosensing by Tandem Polymerizatio and Cleavage-Mediated Cascade Target Recycling and DNAzym Amplificatio, *Biosensors and Bioelectronic* http://dx.doi.org/10.1016/j.bios.2015.10.056

Label-free Electrochemical Nucleic Acid Biosensing by Tandem Polymerization and Cleavage-Mediated Cascade Target Recycling and DNAzyme Amplification

Shufeng Liu*, Hongwei Gong, Yanqun Wang, and Li Wang*

Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, No. 53 Rd. Zhengzhou, Qingdao, Shandong 266042, China

Revised to ***Biosensor and Bioelectronics***

---

* Corresponding author.

Tel. &Fax. 86-532-84022681. E-mail address: sliu@qust.edu.cn (S. Liu); wangli@qust.edu.cn (L. Wang)

Abstract:

Owing to the intrinsic importance of nucleic acid as bio-targets, the achievement of its simple and sensitive detection with high confidence is very essential for biological studies and diagnostic purposes. Herein, a label-free, isothermal, and ultrasensitive electrochemical detection of target DNA was developed by using a tandem polymerization and cleavage-mediated cascade target recycling and DNAzyme releasing amplification strategy. Upon sensing of the nucleic acid analyte for the assembled hairpin-like probe DNA on the electrode, the DNA polymerase guided the target recycling and simultaneously triggered the lambda exonuclease cleavage, accompanied by the cascade recycling of the released new complementary strand and the amplified liberation of the G-rich sequence of the HRP-mimicking DNAzyme. The electrocatalytic reduction of H_2O_2 by the generated hemin/G-quadruplex DNAzyme was used for the signal readout and further amplification toward target response. Such tandem functional operation by DNA polymerase, lambda exonuclease and DNAzyme endows the developed biosensor with a high sensitivity and also a high confidence. A low detection limit of 5 fM with an excellent selectivity toward target DNA could be achieved. It also exhibits the distinct advantages of simplicity in probe design and biosensor fabrication, and label-free electrochemical detection, thus may offer a promising avenue for the applications in disease diagnosis and clinical biomedicine.

Keywords: electrochemical biosensor; DNA; target recycling; DNAzyme

1. Introduction

The development of ultrasensitive DNA biosensing platform to profile low abundance of target DNA is being greatly motivated by its potential applications in the early diagnosis and clinical treatment for some major diseases such as cancers (Sassolas et al., 2008; Zhao et al., 2014; Turner, 2013; Song et al., 2010). The polymerase chain reaction (PCR) may be the most widely used strategy for the amplified analysis of nucleic acid, but it involves the precise control of temperature cycling and the sophistication of primer design (Sanchez-Freire et al., 2012; Chen et al., 2005). Alternatively, the autocatalytic DNA biosensor or DNA-based machine has become the attractive tools owing to its easy operation, isothermal reaction, and high sensitivity (Weizmann et al., 2008; Wang et al., 2012; Bi et al., 2014; Wang et al., 2011a). For example, the autonomous replication-scission-displacement strategy operated by polymerase and nicking endonuclease could offer a prominent amplification capability for nucleic acid detection (Jia et al., 2010; Zhuang et al., 2014; Yin et al., 2013; Weizmann et al., 2006; Yu et al., 2014). However, the analyzed nucleic acid is directly used as trigger for the DNA replication-scission-displacement reaction process, which thus limits the detection universality and specificity of the fabricated biosensor. The autocatalytic DNA biosensors based on the sole or combined use of nucleases for example exonuclease, endonuclease, etc have also been proposed (Duan et al., 2013; Liu et al., 2015; Zhu et al., 2014). But they may be confronted with some disadvantages including the relatively complex detection or probe design procedures, and sometimes unsatisfactory detection performance. Furthermore, the autocatalytic DNA biosensors are more preferentially combined with fluorescence signal transduction means. By comparison, electrochemical methods can provide significant advantages, such as inherent signal stability, low cost, high

sensitivity and ease of calibration (Ronkainen et al., 2010; Hsieh et al., 2010; Tang et al., 2014; Liu et al., 2014). Currently, the conventional electrochemical DNA biosensors are usually associated with the electroactive labels for the electrical signal readouts. The electroactive labels could be introduced by direct coupling toward the probe DNA or by the further recognition events after target response, which indicates the addition of some exogenous reagents or procedures for the electrical signal readouts. Thus, the design of convenient, isothermal and autonomous amplification system for the label-free and sensitive electrochemical detection of nucleic acid is high desirable.

DNAzymes have been widely applied as amplifying labels for bio-recognition or biosensing events. They are a variety of short DNA sequences selected by SELEX that show high catalytic activities toward the specific substrates (Liu and Lu, 2007; Kim, et al., 2007; Wang et al., 2014; Hu et al., 2015). For example, metal-dependent DNAzymes have been widely used as the recognition unit for the detection of metal ions, and also as the signal transduction unit for the detection of more targets for example nucleic acid, protein and etc (Gong et al., 2015; Zhao et al., 2013; Freage et al., 2014; Lu et al., 2011; Hwang et al., 2014; Xiao et al., 2015; Torabi et al., 2015). The hemin/G-quadruplex DNAzyme possesses the horseradish peroxidase-mimicking activity, and may be the most frequently used biocatalytic DNAzyme label for amplified biosensing. Till now, the hemin/G-quadruplex DNAzyme as amplifying label has been well explored for the fabrication of optical or chemiluminescence biosensors (Tang et al., 2013; Shimron et al., 2012; Zong et al., 2014; Wang et al., 2011b; Gao and Li, 2014; Zhou et al., 2014; Mun et al., 2014; Zheng et al., 2012). However, the use of hemin/G-quadruplex DNAzyme as electrocatalytic labels for amplifying sensing events is still underexplored. Willner et al., originally utilized the

HRP-mimicking DNAzyme as the electrocatalyst for the electrochemical biosensor fabrication (Pelossof et al., 2010; Pelossof et al., 2012). Some following endeavors have also been made to explore the hemin/G-quadruplex DNAzyme for the sensitive detection of various targets (Yuan et al., 2015; Chen et al., 2011; Deng et al., 2014; Wu et al., 2015). But the development of label-free DNAzyme-based electrochemical assays with further upgraded detection performance still remains a major and urgent challenge for the potential applications in disease diagnosis and clinical biomedicine.

Herein, a tandem polymerization and cleavage-mediated cascade target recycling and DNAzyme amplification strategy was proposed for the label-free and ultrasensitive detection of target DNA. A hairpin-like probe (HP) was designed and immobilized onto the electrode. It contained the recognition sequence for target DNA in the loop region and the partially caged G-rich sequence of the HRP-mimicking DNAzyme in the stem region. The DNA polymerase guided the primer elongation for the target recycling and the generation of new duplex DNA. Then, the lambda exonuclease could be triggered to digest the HP strand from its 5'-phosphate termini for the releasing of the corresponding complementary strand, which could be used as secondary target for the successive lamda exonuclease cleavage toward HP. By following the current tandem polymerization and cleavage-mediated cascade target recycling strategy, the caged G-rich sequence of the HRP-mimicking DNAzyme could be fully liberated to play its electrocatalytic role toward H_2O_2 reduction for the electrochemical signal readout. The current strategy could be used to detect DNA down to the 5 fM level and could discriminate mismatched DNA from perfectly matched target DNA, which thus holds a great potential in detecting nucleic acids with low abundance in bioanalysis and clinical biomedicine.

2. Experimental section

2.1 Chemicals and reagents

6-Mercaptohexanol (MCH) and hemin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Klenow fragment polymerase (3′ → 5′exo-, KF polymerase) and the lambda exonuclease were obtained from New England BioLabs (Beverly, MA, USA). dNTPs, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES) and fetal bovine serum were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Tris(hydroxymethyl)aminomethane (Tris) and dimethyl sulfoxide (DMSO) was purchased from Sinopharm Chemical Regent Co., Ltd (Shanghai, China). All other chemicals were of analytical grade and used as received. A hemin stock solution in DMSO was stored in dark at -20 °C. The HPLC-purified oligonucleotide sequences are purchased from Sangon Biotech. Co., Ltd. (Shanghai, China) and listed in Table S1. The synthetic target DNA sequence was associated with the breast cancer gene, BRCA1.

2.2 Electrode Pretreatment

The gold electrode was cleaned by immersion in a freshly prepared piranha solution (a 3:1 v/v mixture of concentrated H_2SO_4 and 30% H_2O_2) for 20 min, followed by a thoroughly rinse with ultrapure water. Then the electrode was polished on a microcloth (Shanghai Chenhua Inc., China) with 50 nm alumina slurry to obtain a mirror surface, followed by sonication in acetone and ultrapure water for 5 min each, to remove residual alumina powder. The well-polished electrode was then subjected to electrochemical pretreatment by cycling the potential between -0.2 and 1.6V in H_2SO_4 (0.5 M) at a scan rate of 100 mV s^{-1} until a stable cyclic voltammogram was

obtained, and then the cleaned electrode was allowed to be dried at room temperature.

2.3 Immobilization of hairpin DNA probe (HP) on Au Surface

The assembly of hairpin DNA probe (HP) on the electrode surface was carried out according to the following procedures. The HP was firstly heated to 90 °C for 5 min and then allowed to cool to room temperature for at least 2 hr before use. Then, the cleaned gold electrode was incubated into 50 μL of 1 μM HP in 300 mM Tris-HCl buffer (1.0 M NaCl, 1mM EDTA, 10 mM TCEP, pH 7.4) for 12 h at room temperature and then thoroughly rinsed with 20 mM Tris-HCl buffer (pH 7.4) and ultrapure water, and dried under a stream of nitrogen gas. The electrode was subsequently immersed in 1 mM MCH solution for 1 hour to remove the nonspecific DNA adsorption. Then the electrode surface was rinsed thoroughly and dried in nitrogen.

2.4 The tandem polymerization and cleavage-mediated cascade target recycling process

The target DNA recognition and the following polymerization and cleavage-mediated target recycling experiments were performed with the incubation of HP assemblied electrode into 50 μL 20 mM Tris-HCl buffer (140 mM NaCl, 5 mM $MgCl_2$, pH 7.4) containing 10 U KF polymerase, 8 U lambda exonuclease, 1 μM primer, 300 μM dNTPs and various concentration of target DNA. All reactions were performed at 37 ^{o}C for 90 min (except for the time-course study). Then, the electrode was thoroughly rinsed and dried in nitrogen.

2.5 Electrochemical measurements and apparatus

All electrochemical measurements were carried out by using a CHI 660D electrochemical workstation (CH Instruments, Shanghai, China) at room temperature. A conventional three-electrode system was used, which comprised a gold working electrode (2 mm diameter), a platinum wire auxiliary electrode, and a saturated

calomel reference electrode (SCE). The cyclic voltammograms (CV) were recorded in 10 mM HEPES buffer (pH 7.2, 150 mM NaCl, 50 mM KCl, 1 μM hemin and 2mM H_2O_2) with the scan rate of 10 mV/s. The electrochemical impedance spectra (EIS) experiments were measured in 1 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.5 M KCl with the frequency range from 0.1 Hz to 10 kHz. Before measurements, the electrolyte solution should be thoroughly purged with high purity nitrogen for about 30 min, to avoid the interference from the reduction of oxygen.

3. Results and discussions

3.1 Design principle of the sensor

In this work, a tandem polymerization and cleavage-mediated cascade target recycling and DNAzyme amplification strategy was proposed for electrochemical DNA detection. The electrochemical DNA biosensor fabrication was schematically illustrated in Scheme 1. The synthetic 25-mer oligonucleotide related to BRCA1 gene was used as the model target DNA analyte. A hairpin-like DNA probe (HP) with the thiol group functionalization at 3'-end was designed and assembled on the electrode surface by Au-S interaction. The HP includes the G-rich sequence at the 3'-end (green color), which could form G-quadruplex to be used as the horseradish peroxidase (HRP)-mimicking DNAzyme. The partial sequence for such HRP-mimicking DNAzyme unit is caged in the stem region by its complementary sequence in HP and thus the activity of DNAzyme is inhibited. The DNA segment in the loop region of the HP is used as the recognition unit for target DNA (violet color). The 5'-end of the HP is 5-base overhung and terminated with a phosphate group. The HP itself could not be recognized and cleaved by lamda exonuclease since the preferred substrate for lambda exonuclease is 5'-phosphorylated double-stranded DNA with a blunt 5'-terminus (Zhou et al., 2014; Zhu et al., 2014; Xu et al., 2012). In the presence of

the target DNA, it can hybridize with the loop region of HP and make the HP stem open. Then, an engaging primer anneals with the exposed stem and allows polymerization induced by KF polymerase, which displaces the target DNA and synthesizes a DNA duplex according to the HP probe. The displaced target DNA is free to bind to another HP on the electrode, initiating a new cycle. With each reaction cycle, the target DNA is regenerated and a duplex DNA probe is formed. This constitutes the recycling I stage in Scheme 1. Furthermore, the new synthesized DNA duplex makes the phosphate-labeled 5'-terminus blunt, which can be recognized by lambda exonuclease and then the 5'-phosphorylated strand of the synthesized duplex DNA can be catalytically digested, releasing the corresponding complementary strands. Importantly, the released complementary strands can directly hybridize with the HP on the electrode surface to form complementary DNA duplexes, which can be further recognized and cleaved by lambda exonuclease to recycle the complementary strands. Accompanying with the lambda exonuclease cleavage, the G-rich sequence segment of the HP could be left on the electrode. This constitutes the recycling II stage as indicated in Scheme 1. By following the mechanism, the target DNA can lead to two independent recycling processes, which results in a cascade amplification format for the ultimate catalytic releasing of the G-rich sequence of the HRP-mimicking DNAzyme. The hemin/G-quadruplex HRP-mimicking DNAzyme structures are generated on the electrode surfaces with the help of K^+ and hemin. The electrocatalytic cathodic currents generated by the electrochemical reduction of H_2O_2 offers a quantitative measure for the detection of the target DNA (Pelossof et al., 2010; Pelossof et al., 2012). In the absence of the target DNA, the HP can't be opened and the hemin/G-quadruplex DNAzyme structures can't be generated for the achievement of electrocatalytic reduction of H_2O_2. In the current strategy, the tandem

polymerization and cleavage reaction driven by KF polymerase and lambda exonuclease, respectively, contributes to the ultimate DNAzyme liberation, thus providing a confidential detection toward target DNA. Also, the triplex amplification mode includes recycling I, II and DNAzyme amplification would endow an ultrahigh sensitivity for the assay of nucleic acid. Furthermore, the current biosensor design could be easily extended for the detection toward different target DNA analytes only by simply changing the recognition sequence in the loop region of the HP.

<<Herein Scheme 1>>

3.2 Electrochemical characterization of fabricated DNA biosensor

To verify the feasibility of the designed tandem polymerization and cleavage-mediated cascade target recycling and DNAzyme amplification strategy for target DNA detection, the cyclic voltammograms for the electrocatalytic reduction of H_2O_2 recorded at the different experimental conditions were shown in Figure 1A. In the case of no target DNA and enzymes, the G-quadruplex sequence was caged in the HP1 and almost no active hemin/G-quadruplex DNAzyme could be generated and only a very low electrocatalytic cathodic current could be observed (curve a in Figure 1A). With the introduction of KF polymerase and lambda exonuclease but no target DNA, the electrocatalytic cathodic current corresponding to the background signal was observed with a slight increase (curve b in Figure 1A). The further control experiment in the presence of target DNA but no enzymes demonstrated an increased electrocatalytic cathodic current (curve c in Figure 1A), indicated that the target DNA could open the HP1 for the generation of some active hemin/G-quadruplex DNAzyme. Again, in the presence of lambda exonuclease but no KF polymerase, the electrocatalytic current was not observed with an evident increase (curve d in Figure 1A). Furthermore, an obvious current increase could be obtained in the case of KF

polymerase but no lambda exonuclease (curve e in Figure 1A) than that in the case of only target DNA. This indicated that the target DNA could be recycled by KF polymerase for the opening of HP1 and the generation of more amounts of active hemin/G-quadruplex DNAzyme for the increased electrocatalytic current. However, only a remarkable electrocatalytic cathodic current could be obtained in the coexistence of target DNA, KF polymerase and lambda exonuclease (curve f in Figure 1A), strongly evidenced the signal amplification ability of the tandem polymerase and lambda exonuclease-mediated cascade target recycling strategy for target DNA detection. In current strategy, the phosphate group-functionalized HP1 is necessary for the signal amplification capability played by lambda exonuclease, the preferred substrate of which is 5'-phosphorylated double stranded DNA. We also synthesized a HP2 with no phosphate group label at the 5'-end as a substitute of HP1 for target DNA detection. It could be seen from Figure S1 that the electrocatalytic cathodic current for target DNA detection by HP2 was significantly lower than that by HP1.

The biosensor fabrication and recognition process has also been confirmed by EIS measurements. Figure 1B shows the nyquist plots for the electrode modification at different stages. Obviously, an almost straight line was observed for the bare gold electrode (curve a in Figure 1B), which was the typical characteristics of a diffusion-controlled electron transfer process. The immobilization of HP1 monolayer on the electrode induced a big semicircle with charge transfer resistance (Rct) value of about 4777 Ω (curve b in Figure 1B), which could be ascribed to the repellence of negatively charged redox species, $Fe(CN)_6^{3-/4-}$, from approaching electrode surface by negative-charged phosphate skeletons of HP1. After recognition by target DNA in the presence of KF polymerase and lambda exonuclease, the diameter of the semicircle

decreased dramatically with Rct value of about 2447 Ω (curve c in Figure 1B) since the assembled HP1 on the electrode had been transformed into the short single-stranded G-rich sequence, which was accompanied by the decrease of negative charge and steric hindrance of electrode interface for the enhanced interfacial charge transfer. Furthermore, when the electrode was treated with K^+ and hemin, the Rct value was observed with a further increase to be about 6113Ω compared with that of HP1 assembled electrode (curve d in Figure 1B). This might be explained that the formed G-quadruplex-hemin complexes made a negatively charged interface on the electrode surface to pack more orderly and densely, repelling the negatively charged redox species strongly.

<<Herein Figure 1>>

3.3 Optimization of experimental conditions

In order to achieve the best sensing performance, the corresponding experimental conditions were optimized. The assembly density of the original HP1 probe on the electrode surface would exert an important effect on the performance of electrochemical DNA biosensor. The immobilization concentration of HP1 was firstly examined based on the electrochemical response toward target DNA detection. It could be seen from Figure 2A that the immobilization concentration of HP1 of 1.0 μM could achieve the better electrochemical response than that at other concentrations. The higher immobilization concentration of HP1 may increase the assembly density on the electrode surface, which further may influence the hybridization efficiency of target DNA to the HP1 owing to the steric hindrance effect. The employed amounts of KF polymerase and lambda exonuclease were also optimized. The optimized KF polymerase amount was chosen as 10 U (Figure 2B). The lambda exonuclease amount was optimized as 8 U (Figure 2C). The further increased amount of lambda

exonuclease induced an adverse effect for the electrochemical response toward target DNA detection. The time responses of the tandem polymerization and cleavage-mediated cascade target recycling strategy for target DNA detection was also optimized (Figure 2D). The electrocatalytic cathodic current increased with time and almost reached saturation at about 1.5 h. Thus, the employed reaction time was chosen as 1.5 h.

<<Herein Figure 2>>

3.4 Detection sensitivity of the developed DNA biosensor

To further confirm the ability of the proposed biosensor to sensitively detect target DNA, a series of different concentrations of target DNA ranged from 0 to 0.1 nM were measured (Figure 3A). The electrocatalytic cathodic current increased with the concentration of target DNA, indicating that the formation of the active hemin/G-quadruplex DNAzyme on the electrode surface was highly dependent on the concentration of target DNA. Figure 3B showed the calibration curve of the electrocatalytic cathodic current at -0.6 V with the target DNA concentration. A linear relationship between the electrocatalytic cathodic current and the target DNA concentration ranged from 5 fM to 300 fM could be obtained with the correlation coefficient of 0.9965 (inset of the Figure 3B). The detection limit, which was defined as 3 times the standard deviation of the background, was about 5 fM. This detection limit was superior or comparable to most of the reported electrochemical DNA biosensors (Table S2).

<<Herein Figure 3>>

To further prove the signal amplification effect of the developed tandem polymerization and cleavage-mediated cascade target recycling and DNAzyme amplification strategy, the detection of target DNA was also conducted in the case of

no lambda exonuclease. This indicated that only DNA polymerization contributed for the target recycling and the liberation of DNAzyme. The electrochemical response also increased with the concentrations of target DNA ranged from 0 to 500 pM (Figure S2A). The calibration curve for the electrocatalytic cathodic current with target DNA concentration was shown in Figure S2B. A detection limit of 0.5 pM for target DNA could be only obtained. Also, the tandem polymerization and cleavage-mediated cascade target recycling strategy by using two enzymes could realize a more rapid electrochemical response before reaching equilibrium compared with that in the absence of lambda exonuclease, indicating again that the cascade amplification process for target DNA by using two enzymes indeed occurs (Figure 2D).

3.5 Selectivity, stability of the developed DNA biosensor

The detection specificity of the proposed DNA biosensor was further investigated by using four kinds of DNA sequences, including complementary target DNA (TD), single-base mismatched DNA (1MT), three-bases mismatched DNA (3MT), and non-complementary DNA (NC) (Figure 4). After substracting the blank response, the electrocatalytic cathodic current response toward 1MT and 3MT was about 36.2%, and 11.6% of that for perfect target DNA, respectively. The NC only showed very slightly increased electrochemical response compared with the blank solution. Thus, the developed sensing platform demonstrates a good selectivity for target DNA and the potential to detect mutations efficiently.

<<Herein Figure 4>>

The stability for the HP assembled electrode has also been checked. Three independent experiments demonstrated that the HP assembled electrode could retain about 95.6% of its initial response toward target DNA after its storage in the

refrigerator at 4 °C for two weeks, indicating a relatively robust stability of the modified electrode. In order to further evaluate the practical utility and reliability of the proposed DNA biosensor, we further challenged the detection toward target DNA spiked in a relatively complex biological matrix (5% fetal bovine serum). Comparable responses were obtained for the detection of three different concentrations of target DNA in both buffer and diluted serum (Figure S3), indicating that the developed electrochemical biosensor could be successfully applied to detect target DNA in a relatively complex biological sample.

Conclusions

Conclusively, we have developed a label-free, isothermal electrochemical platform for ultrasensitive DNA detection using a tandem polymerization and cleavage-mediated cascade target recycling and DNAzyme amplification strategy. It could achieve the detection of target DNA down to the 5 fM level and could discriminate mismatched DNA from perfectly matched target DNA. The advised tandem enzyme functional operation endows the biosensor with a high sensitivity and also guarantees the target profiled with a high confidence. Furthermore, the developed protocol also demonstrates the distinct advantages including the simplicity in probe design and biosensor fabrication, and label-free electrochemical detection. It should be fairly easy to extend for the detection of various targets. Therefore, it opens a promising avenue to develop label-free and ultrasensitive electrochemical biosensors and should be a very beneficial supplement for the current biosensor scope.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (No. 21475072), the Natural Science Foundation of Shandong Province of China (Nos. ZR2015JL007, ZR2014BM019), and the Basic Research Program of Qingdao (No. 13-1-4-214-jch).

References

Bi, S., Cui, Y., Dong, Y., Zhang, N., 2014. Biosens. Bioelectron. 53, 207-213.

Chen, C., Ridzon, D. A., Broomer, A. J., Zhou, Z., Lee, D. H., Nguyen, J. T., Barbisin, M., Xu, N. L., Mahuvakar, V. R., Andersen, M. R., Lao, K. Q., Livak, K. J., Guegler, K. J., 2005. Nucleic Acids Res. 33, e179.

Chen, J., Zhang, J., Guo, Y., Li, J., Fu, F., Yang, H. H., Chen, G., 2011. Chem. Commun. 47, 8004-8006.

Deng, H., Shen, W., Ren, Y., Gao, Z., 2014. Biosens. Biosens. 60, 195-200.

Duan, R., Zuo, X., Wang, S., Quan, X., Chen, D., Chen, Z., Jiang, L., Fan, C., Xia, F., 2013. J. Am. Chem. Soc. 135, 4604-4607.

Freage, L., Wang, F., Orbach, R., Willner, I., 2014. Anal. Chem. 86, 11326-11333.

Gao, Y., Li, B., 2014. Anal. Chem. 86, 8881-8887.

Gong, L., Zhao, Z., Lv, Y. F., Huan, S. Y., Fu, T., Zhang, X. B., Shen, G. L., Yu, R. Q., 2015. Chem. Commun. 51, 979-995.

Hsieh, K., Xiao, Y., Soh, H.T., 2010. Langmuir 26, 10392-10396.

Hu, L., Lu, C. H., Willner, I., 2015. Nano Lett. 15, 2099-2103.

Hwang, K., Wu, P., Kim, T., Lei, L., Tian, S., Wang, Y., Lu, Y., 2014. Angew. Chem., Int. Ed. 53, 13798-13802.

Jia, H.; Li, Z.; Liu, C.; Cheng, Y. Angew. Chem., Int. Ed. 2010, 49, 5498-5501.

Kim, H. K., Liu, J., Li, J., Nagraj, N., Li, M., Pavot, C. M. B., Lu, Y., 2007. J. Am. Chem. Soc. 129, 6896-6902.

Liu, J., Lu, Y., 2007. Angew. Chem. Int. Ed. 119, 7731-7734.

Liu, S., Cheng, C., Liu, T., Wang, L., Gong, H., Li, F., 2015. Biosens. Bioelectron. 63, 99-104.

Liu, S., Lin, Y., Liu, T., Cheng, C., Wei, W., Wang, L., Li, F., 2014. Biosens.Bioelectron. 56, 12-18.

Lu, L. M., Zhang, X. B., Kong, R. M., Yang, B., Tan, W., 2011. J. Am. Chem. Soc. 133, 11686-11691.

Mun, H., Jo, E. J., Li, T., Joung, H. A., Hong, D. G., Shim, W. B., Jung, C., Kim, M. G., 2014. Biosens. Bioelectron. 58, 308-313.

Pelossof, G., Tel-Vered, R., Elbaz, J.,Willner, I., 2010. Anal. Chem. 82, 4396-4402.

Pelossof, G., Tel-Vered, R., Willner, I., 2012. Anal. Chem. 84, 3703-3709.

Ronkainen, N. J., Halsall, H. B., Heineman, W. R., 2010. Chem. Soc. Rev. 39, 1747-1763.

Sanchez-Freire, V., Ebert, A. D., Kalisky, T., Quake, S. R., Wu, J. C., 2012. Nat. Protoc. 7, 829-838.

Sassolas, A., Leca-Bouvier, B. D., Blum, L. J., 2008. Chem. Rev. 108, 109-139.

Shimron, S., Wang, F., Orbach, R., Willner, I., 2012. Anal. Chem. 84, 1042-1048.

Song, S., Qin, Y., He, Y., Huang, Q., Fan, C., Chen, H. Y., 2010. Chem. Soc. Rev. 39, 4234-4243.

Tang, S., Tong, P., Li, H., Gu, F., Zhang, L., 2013. Biosens. Bioelectron. 41, 397-402.

Tang, Y., Ge, B., Sen, D., Yu, H. Z., 2014. Chem. Soc. Rev. 43, 518-529.

Torabi, S. F., Wu, P., McGhee, C. E., Chen, L., Hwang, K., Zheng, N., Cheng, J., Lu, Y., 2015. Proc. Natl. Acad. Sci. USA 112, 5903-5908.

Turner, A. P. F., 2013. Chem. Soc. Rev. 42, 3184-3196.

Wang, F., Elbaz, J., Teller, C., Willner, I., 2011b. Angew. Chem., Int. Ed. 50, 295-299.

Wang, F., Elbaz, J., Willner, I., 2012. J. Am. Chem. Soc. 134, 5504-5507.

Wang, H. Q., Liu, W. Y., Wu, Z., Tang, L. J., Xu, X. M., Yu, R. Q., Jiang, J. H., 2011a. Anal. Chem. 83, 1883-1889.

Wang, L., Liu, Y., Li, J., 2014. Anal. Chem. 86, 7907-7912.

Weizmann, Y., Beissenhirtz, M. K., Cheglakov, Z., Nowarski, R., Kotler, M., Willner, I., 2006. Angew. Chem., Int. Ed. 45, 7384-7388.

Weizmann, Y., Cheglakov, Z., Willner, I., 2008. J. Am. Chem. Soc. 130, 17224-17225.

Wu, H., Zhang, K., Liu, Y. L., Wang, H. Y., Wu, J., Zhu., F. F., Zou, P., 2015. Biosens. Bioelectron. 64, 572-578.

Xiao, S. J., Zuo, J., Zhu, Z. Q., Ouyang, Y. Z., Zhang, X. L., Chen, H. W., Zhang, L., 2015. Sensor. Actuat. B: Chem. 210, 656-660.

Xu, H., Wang, L., Ye, H., Yu, L., Zhu, X., Lin, Z., Wu, G., Li, X., Liu, X., Chen, G., 2012. Chem. Commun. 48, 6390-6392.

Yin, B. C., Liu, Y. Q., Ye, B. C., 2013. Anal. Chem. 85, 11487-11493.

Yu, Y., Chen, Z., Shi, L., Yang, F., Pan, J., Zhang, B., Sun, D., 2014. Anal. Chem. 86, 8200-8205.

Yuan, L., Tu, W., Bao, J., Dai, Z., 2015. Anal. Chem. 87, 686-692.

Zhao, W. W., Xu, J. J., Chen, H. Y., 2014. Chem. Rev. 114, 7421-7441.

Zhao, X. H., Gong, L., Zhang, X. B., Yang, B., Fu, T., Hu, R., Tan, W., Yu, R., 2013. Anal. Chem. 85, 3614-3620.

Zheng, A. X., Li, J., Wang, J. R., Song, X. R., Chen, G. N., Yang, H. H., 2012. Chem. Commun. 48, 3112-3114.

Zhou, W., Gong, X., Xiang, Y., Yuan, R., Chai, Y., 2014. Anal. Chem. 86, 953-958.

Zhu, G., Liang, L., Zhang, C. Y., 2014. Anal. Chem. 86, 11410-11416.

Zhuang, J., Tang, D., Lai, W., Chen, G., Yang, H., 2014. Anal. Chem. 86, 8400-8407.

Zong, C., Wu, J., Liu, M., Yang, L., Yan, F., Ju, H., 2014. Anal. Chem. 86, 9939-9944.

Figure Captions

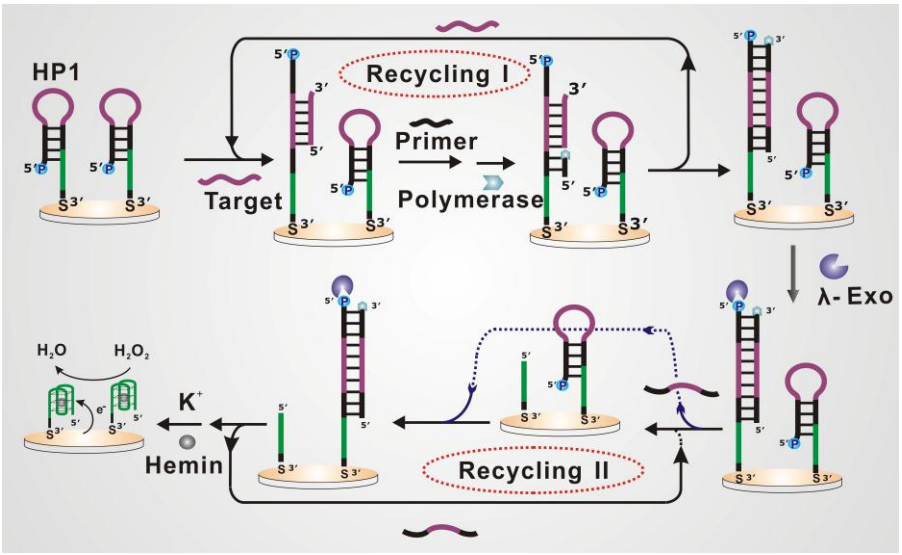
Scheme 1 : Schematic illustration of the tandem polymerization and cleavage-mediated cascade target recycling and DNAzyme amplification strategy for DNA assay.

Figure 1. (A) Cyclic voltammograms corresponding to the electrocatalytic reduction of H_2O_2 obtained at different experimental conditions. (a) no target, no enzymes; (b) KF polymerase + lambda exonuclease; (c) 10 pM target DNA, no enzymes; (d) 10 pM target DNA + lambda exonuclease; (e) 10 pM target DNA + KF polymerase; (f) 10 pM target DNA + KF polymerase + lambda exonuclease. The concentrations for the KF polymerase and lambda exonuclease were 10 U and 8 U, respectively. Inset shows the bar chart of the electrocatalytic currents obtained at E = -0.6 V vs SCE for the different experimental conditions. (B) Nyquist diagrams for the electrochemical impedance measurements of the gold electrode at different modification stages. Curve (a) is for the bare gold electrode, while curves (b), (c) and (d) are the cases after HP1 assembly, treatment by target DNA, KF polymerase and lambda exonuclease, and the formation of G-quadruplex-hemin complex with the help of K^+ and hemin, respectively.

Figure 2. Optimization of experimental conditions. (A) HP immobilization concentration optimization. (B) Optimization of KF polymerase amount. (C) Optimization of lambda exonuclease amount. (D) The time response of fabricated biosensor toward target DNA detection in the presence of KF polymerase and lambda exonuclease (square), and absence of lambda exnoclease (circle). The used target DNA concentration is 10 pM.

Figure 3. (A) Cyclic voltammograms corresponding to analysis of different concentrations of target DNA. The concentrations of target DNA for the curves (a) to (l) are: (a) 0, (b) 5 fM, (c) 20 fM, (d) 50 fM, (e) 100 fM, (f) 200 fM, (g) 300 fM, (h) 500 fM, (i) 1pM, (j) 5 pM, (k) 10 pM, and (l) 0.1 nM. (B) Calibration curve corresponding to the electrocatalytic cathodic current measured at -0.6 V for variable concentration of target DNA. Error bars represent standard deviations of measurements (n=3). Inset shows the linear relationship between the electrocatalytic cathodic current and the target DNA concentration.

Figure 4. (A) Cyclic voltammetric responses toward the blank (a) and four various DNA sequences including non-complementary DNA (NC) (b), three-bases mismatched DNA (3MT), (c), single-base mismatched DNA (1MT) (d), and complementary target DNA (TD) (e). The concentrations of various DNA sequences were all 0.1 nM.



Scheme 1

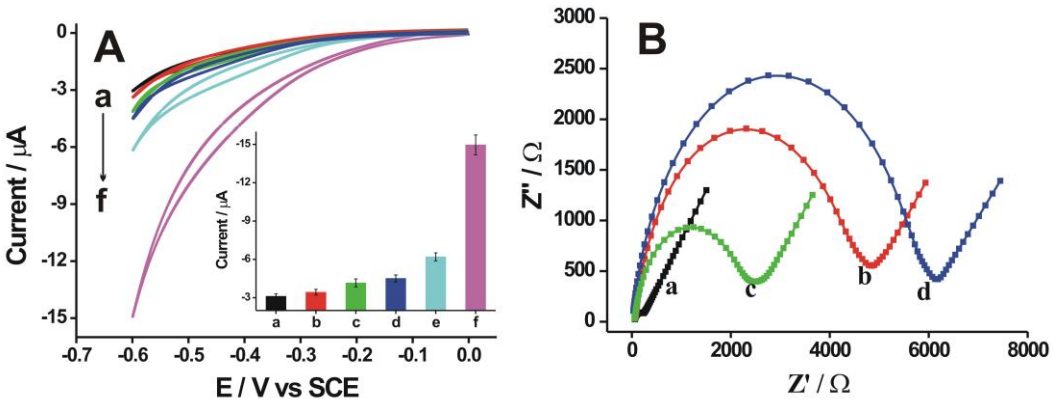


Figure 1

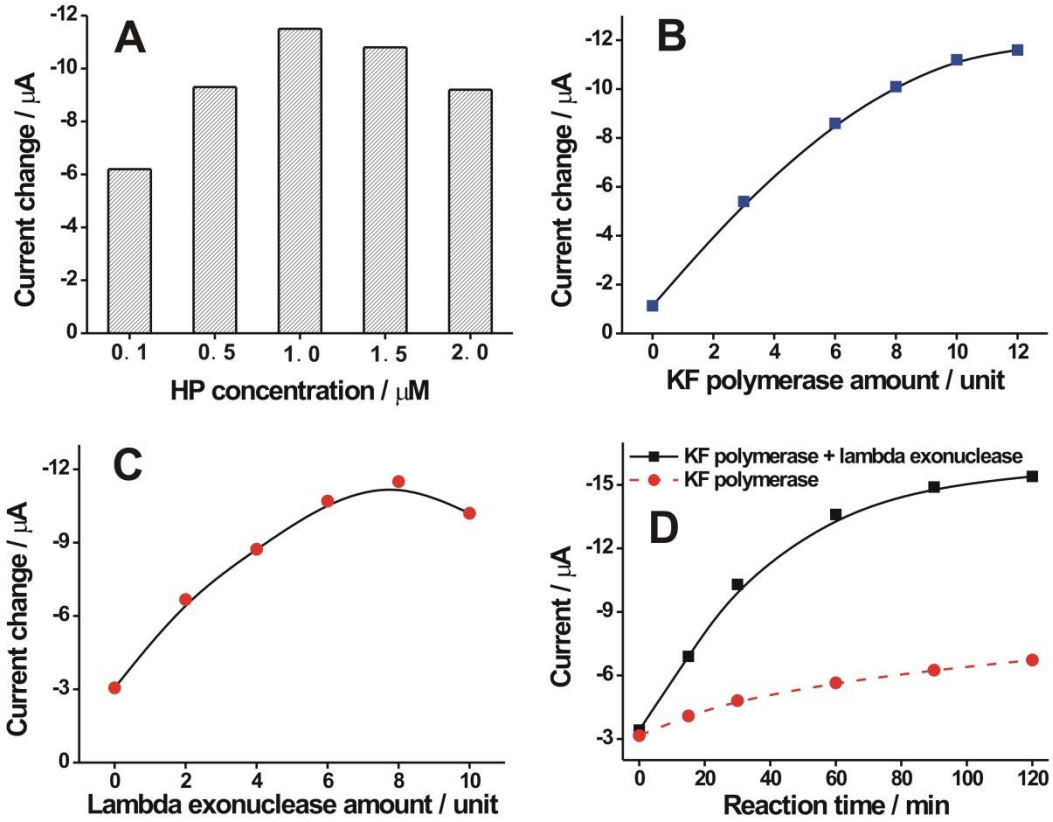


Figure 2

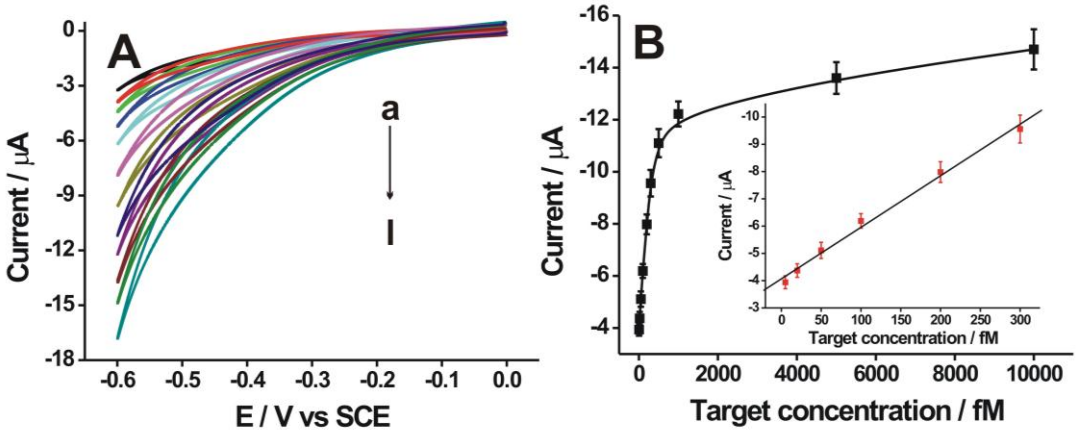


Figure 3

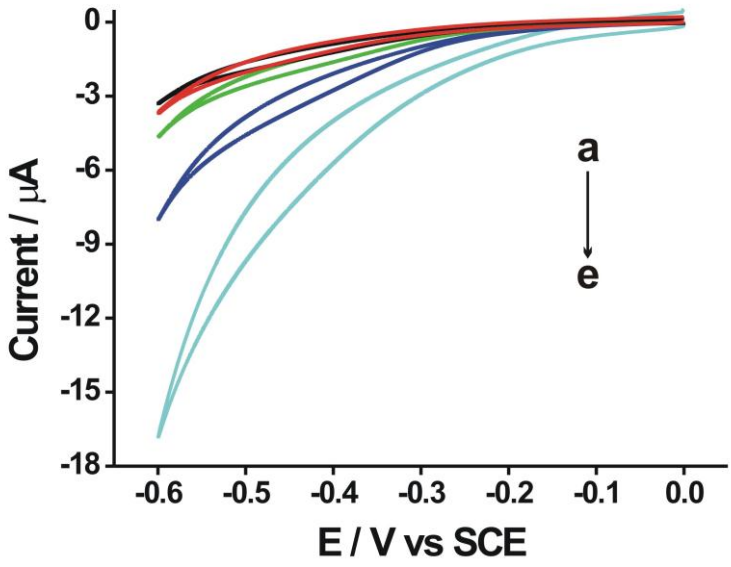


Figure 4

Highlights

- A label-free, isothermal and ultrasensitive electrochemical DNA biosensing platform was developed.
- A tandem polymerization and cleavage-mediated target recycling and DNAzyme amplification strategy was proposed.
- A low detection limit of 5 fM toward target DNA with a good selectivity could be achieved.
- It offers a promising avenue for the fabrication of label-free and ultrasensitive electrochemical biosensors.