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ORIGINAL PAPER

A homogeneous electrochemical DNA sensor on the basis of a self-assembled thiol layer on a gold support and by using tetraferrocene for signal amplification

Tianjiao Ning[1] · Fusheng Liao[1] · Hanfeng Cui[1] · Zhaojiang Yin[1] · Guangqiang Ma[1] · Lin Cheng[1] · Nian Hong[1] · Jun Xiong[1] · Hao Fan[1]

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Abstract

An unmodified electrochemical biosensor has been constructed, which can directly detect DNA in homogeneous solution. The synthesized new compound tetraferrocene was used for signal amplification. The dual-hairpin probe DNA was tagged with a tetraferrocene at the 3′ terminal and a thiol at the 5′ terminal. Without being hybridized with target DNA, the loop of probe prevented the thiol from contacting the exposed gold electrode surface with an applied potential. After hybridization with the target DNA, the loop-stem structure of the probe was opened, which led to the formation of the hairpin DNA structure. Afterwards, the thiol easily contacted the electrode and accomplished potential-assisted Au-S self-assembly. Its current signal depends on the concentration of target DNA in the 1.8×10^{-13} to 1.8×10^{-9} M concentration range, and the detection limit is 0.14 pM. The technique is a meaningful study because of its high selectivity and sensitivity.

Keywords DNA sensor; · Homogeneous solution · Tetraferrocene · Au-S self-assembly

Introduction

Deoxyribonucleic acid (DNA) is a medium for genetic information and can be used as a probe to detect target DNA and microRNA. Since DNA can be used as a biological vector to store and transmit genetic information, the effective detection of specific DNA sequences is of particular concern [1–3]. A variety of biosensors, including biosensors based on colorimetry [4, 5], fluorescence [6–8], electrochemical luminescence [9, 10], photoelectrochemistry [11], dynamic light scattering technology [12], and electrochemistry [13–15], have been applied to detect DNA. Among these methods, electrochemical biosensors are extremely attractive because of their excellent sensitivity, low cost, simplicity, and rapidity [16, 17].

The sensitivity of an electrochemical DNA sensor is affected by hybridization efficiency and electroactive indicators. In terms of hybridization efficiency, the vast majority of electrochemical DNA sensors are bound to require probe DNA to be immobilized on the electrode surface [18, 19]. On the one hand, these procedures are time-consuming and reduce reproducibility of the biosensor. On the other hand, DNA hybridization takes place at the solution-electrode interface. This heterogeneous hybridization strategy has significantly low hybridization efficiency due to the spatial hindrance effect of the electrode surface [2, 20]. Homogeneous methods can benefit from the higher binding efficiency and faster reaction rate in solution than in a solid phase compared with heterogeneous assays [21, 22]. Thus, it is crucial to develop a homogeneous immobilization-free system. In terms of electroactive indicators, electrochemical signal markers such as nanoparticles [23–25], metal-organic frameworks (MOFs) [19, 26, 27], and enzymes [21, 28] can transform electroactive indicators into quantitative signal efficiency and achieve signal amplification in DNA assays. However, these materials require additional substances and operations to obtain the desired signal,

Tianjiao Ning and Fusheng Liao contributed equally to this work.

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✉ Jun Xiong
xiongjun196071@163.com

✉ Hao Fan
fanhao11@aliyun.com

[1] Department of Pharmacy, The Affiliated Hospital, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, Jiangxi, China

which overly complicates the operation process making it time-consuming and laborious. Compared with the above electrochemical signal markers, ferrocene, a redox-active substance, can generate a signal directly without additional materials and operation. Therefore, ferrocene is more applicable in the design of electrochemical biosensors. In addition, the electrochemical sensor labeled with ferrocene can not only simplify the operation procedure but also reduce the experimental cost [29–31]. However, since each oxidation-reduction process of ferrocene is only a matter of a gain or loss of single electron, the signal provided for analysis of the target has reduced detection efficiency and sensitivity.

It is worth noting that novel electrochemical signal probes bearing two ferrocene molecules have been successfully studied and synthesized to detect DNA. Xiong et al. developed a DNA probe modified with two ferrocenes at each end to enhance the oxidation signal [16]. Yang et al. synthesized a ferrocene dsDNA intercalator from naphthalene diimide generating a double-ferrocene redox marker to improve the electrical signal [19]. Therefore, the development of the signal of DNA probe of multi-ferrocene is of significant importance. Since multi-ferrocene is an excellent electrochemical signal amplification marker, we designed a double-hairpin DNA probe with a tetraferrocene-labeled 3′ terminal, whereas the 5′ terminal was modified with a thiol. As shown in Fig. 1, prior to hybridization, the thiol was enclosed in the stem-loop structure hindering contact with the gold electrode due to steric effect. After hybridization with the target DNA, the stem-loop structure of the probe opened allowing the thiol to be easily connected to the surface of the bare gold electrode resulting in potential-assisted Au-S self-assembly. And then the tetraferrocene was close enough to the surface of the electrode to produce an excellent signal, which can be used to sensitively quantify DNA.

Experimental section

Materials

Unless otherwise stated, all chemicals were analytical grade reagents purchased from Sangon Biotech Company (Shanghai, China; https://www.sangon.com). Hyperpure water was purchased from a Millipore water purification system (18 MW, Milli-Q, Millipore) and applied to all assays. The oligonucleotide sequences used in this experiment process were purchased from the Sangon Biotech Company, Ltd. (Shanghai, China). In addition, oligodeoxynucleotides were purified by C-18 RP-HPLC. The bound of the tetraferrocene and DNA was accomplished with the help of the Sangon Biotech Company. The probe sequences of probe 1 (P1) and probe 2 (P2) are shown as follows:

P1: 5′-HS-$(CH_2)_6$-CGACAGAGTTTTGTCTGTCG GGCCCCCCTTTTTGGGGGGCCA$_{18}$-tetraferrocene-3′

P2: 5′-HS-$(CH_2)_6$-CGACAGAGTTTTGTCTGTCG GGCCCCCCTTTTTGGGGGGCCA$_{18}$-ferrocene-3′

In addition, the target sequence oligo 3 (5′-CGAC AGACAAAACTCTGTCG-3′) completely matched the partial probe sequence, while oligo 4 (5′-CGACAGACTAAAC TCTGTCG-3′) contained single-base mismatch, oligo 5 (5′-CGACAGACTTTACTCTGTCG-3′) had three base mismatches, and oligo 6 (5′-GCTCAATTCCTTTGGACTAAT-3′) had a random sequence independent of the probe sequence.

oligo 3: 5′-CGACAGACAAAACTCTGTCG-3′
oligo 4: 5′-CGACAGACTAAACTCTGTCG-3′
oligo 5: 5′-CGACAGACTTTACTCTGTCG-3′
oligo 6: 5′-GCTCAATTCCTTTGGACTAAT-3′

Apparatus

All electrochemical measurements were carried out using the auto lab electrochemical workstation (Metrohm Instruments Co, Swiss) at room temperature, including cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A conventional three-electrode cell, which consisted of a polycrystalline gold electrode (1 mm in diameter, working electrode), platinum wire (auxiliary electrode), and Ag/AgCl/KCl (sat) electrode (reference electrode), was used in all electrochemical investigations.

Electrode pretreatment

Gold electrodes were cleaned following a reported procedure. In brief, they were polished with 50-nm aluminum oxide

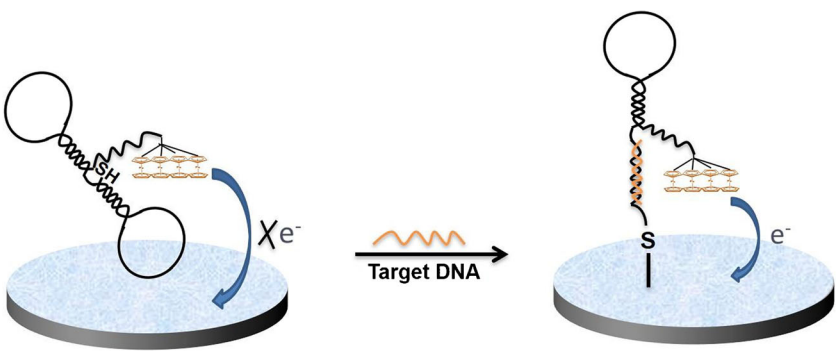

Fig. 1 Scheme of the unmodified electrode electrochemical DNA sensor

particles on a polishing pad for 5 min, followed by sonication in ultrapure water, polishing on a blank polishing pad, and sonication in ultrapure water to remove any particles. Gold electrodes were rinsed with fresh piranha solution (H_2SO_4/H_2O_2, v/v 7/3) followed by rinsing thoroughly with deionized water and readily used for electrochemical cleaning. Electrodes were electrochemically cleaned in a classical three-electrode cell by immersing them in H_2SO_4 (0.5 M) solution. And the potential was scanned between the oxidation and reduction potentials of gold, 0 V and + 1.5 V versus Ag/AgCl reference electrode, with a scanning rate at 0.2 V/s for 60 cycles until no further changes in the voltammogram were observed [19].

Potential-assisted deposition of Au-S on electrode

Potential control is the performer of the DNA self-assembly process [17]. The results show that applying low positive potential on the gold surface will accelerate the chemical adsorption process on its surface and contribute to the tissue monolayer. The cleaned gold electrode was initially run at 0.3 V for 5 min to reduce the gold electrode surface. Afterwards, the electrode was immersed in hybridization solution containing the probe and target DNA, incubated at a potential of 0.4 V for 5 min, and then washed thoroughly with water to remove weakly adsorbed DNA.

Results and discussion

Characterization of the biosensor

Differential pulse voltammetry (DPV) was used to characterize the efficiency of electrochemical bonding on the electrode surface before and after hybridization with the target DNA. In Fig. 2(A), a negligible DPV signal can be observed before hybridization with the target DNA (signal a), which due to the probe double-stem-loop structure was closed with an applied potential, the non-specific adsorption of the probe produced a negligible DPV signal. A highly sensitive electrochemical signal can be observed at around 0.4 V when the signal marker tetraferrocene reached the electrode surface (signal b). This was because the tetraferrocene units were simultaneously oxidized, resulting in a higher peak current peak. In addition, self-assembly at the electrochemical sensor was investigated by electrochemical impedance spectroscopy (EIS). Embedded in Fig. 2(B) is a fitting Randles equivalent circuit of Nyquist diagram. Resistance (Rct) represents interfacial resistance, which is equivalent to the diameter of semicircle in Nyquist diagram. It was observed from the diagram that the bare electrode exhibited a very small semicircle domain (Rct = 230 Ω, curve a), demonstrating an extremely fast charge-transfer process. Subsequently, when the probe was incubated with Au electrode in potential-assisted conditions for 5 min, only a small increase in the semicircle's Rct value of about 456 Ω (curve b) was observed. It indicated that a small amount of probe was non-specifically adsorbed on the electrode surface. Nevertheless, hybridization of the probe with the target DNA at a concentration of 180 pM promoted a large increase of the Rct value (about 1912 Ω, curve c). Hence, a large number of hybrids were immobilized on the electrode surface by Au-S bonding with Au electrode in potential-assisted conditions.

Preparation of probe DNA

A series of researches have been carried out on the design of the probe [21, 22, 32], which is an important factor to determine the response of biosensors. The structural change of the DNA probe influences the reaction performance of the sensor with target DNA. Therefore, we designed the probe with a dual-stem-loop structure, which was verified by Mfold Web Server and fluorescence experiments (see Supporting Information). Fluorescence experiments show that only the double-stem ring structure was thermally stable under our optimized hybridization conditions, which confirmed the applicability of the selected sequences.

In order to make the signal marker on the probe as close as possible to the electrode surface and generate the electrochemical signal, we added a free sequence at the 5′ end and extended the probe design, which had been verified by the experimental process. As shown in Fig. 3, the fluorescence emission spectra had indicated a change in the fluorescence intensity of the probe DNA before (a) and after (b) hybridization with the target DNA, and a significant decrease in the fluorescence intensity after hybridization. The experimental results had verified that the hybridization of the probe with the target DNA resulted in tetraferrocene markers to be close to the electrode surface.

Comparison of signal amplification of tetraferrocene

The signal amplification ability of tetraferrocene as signal markers in the prepared sensor was compared. In the experiment, two probes were placed in the same solution containing target DNA. As shown in Fig. 4, the oxidation peaks of tetraferrocene and ferrocene were at the potential of about 0.4 and 0.2 V, respectively. The electrical signal produced by tetraferrocene was significantly higher than that of ferrocene. The reason is that the four ferrocene units have a higher oxidation potential than ferrocene. Therefore, tetraferrocene as an electrochemical marker generated better signal amplification.

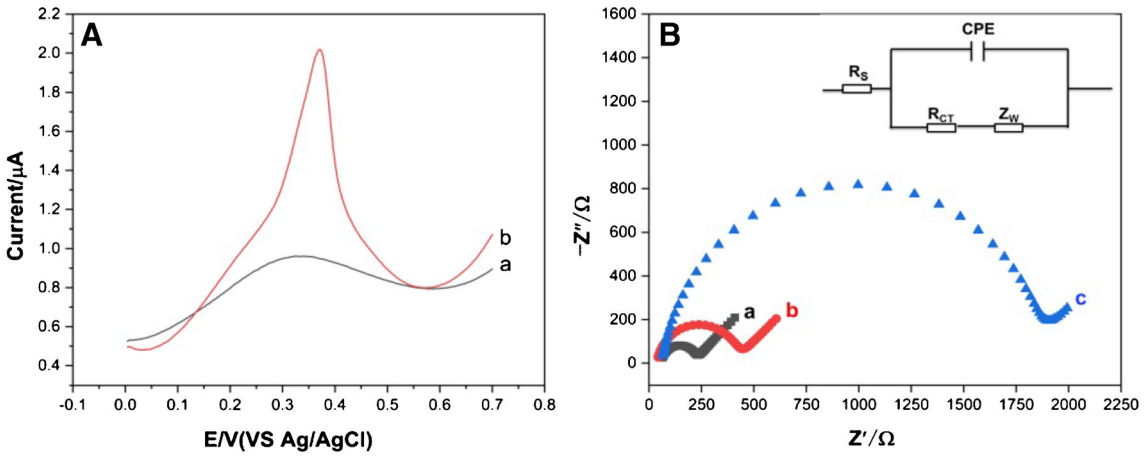

Fig. 2 (A) DPV signal before and after hybridization with target DNA. (B). EIS (Nyquist plots) of the different modification process of the electrode interface in solution containing 5 mM (1:1) $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. Bare electrode (a), probe (b), and probe/target DNA (c). The inset plot shows the equivalent electrical circuit

Optimization of detection conditions

A series of experiments were performed to establish optimal DNA detection conditions, including hybridization temperature and time, Mg^{2+} concentration, and potential-assisted Au-S deposition time (see Supporting Information for further details).

Hybridization temperature of the complementary target DNA incubated with double-hairpin probe ranged between 30 and 51 °C. As shown in Fig. S3, the maximum signal value was at 45 °C, which was used in all consecutive experiments. Hybridization time of 20–60 min was set, and the response signals at different hybridization times are shown in Fig. S4. Upon increasing the hybridization time from 20 to 45 min, a slight increase in signal was observed. However, the signal tended to be stable 45 min. Therefore, the reaction time of 45 min was optimal in the experiment.

The effect of potential-assisted Au-S deposition time on the hybridization experiment is shown in Fig. S5. The current signal value increased with the increase of the deposition time. When the deposition time reached 5 min, the current signal value tended to be stable, and the signal response value of the current hardly changed with the increase of settling time. As a result, the deposition time of 5 min had been selected as an optimal condition.

In the case of the dual-hairpin DNA probe, competition existed between intermolecular target DNA probe hybridization and intramolecular stem part hybridization. Therefore, determination of Mg^{2+} concentration is important, since high Mg^{2+} concentration enhances hybridization between target DNA and double-stem-loop probe. However, excessive concentrations of Mg^{2+} can adversely affect DNA detection performance. Figure S6 shows that the experiment's max signal was at 3-mM Mg^{2+} concentration.

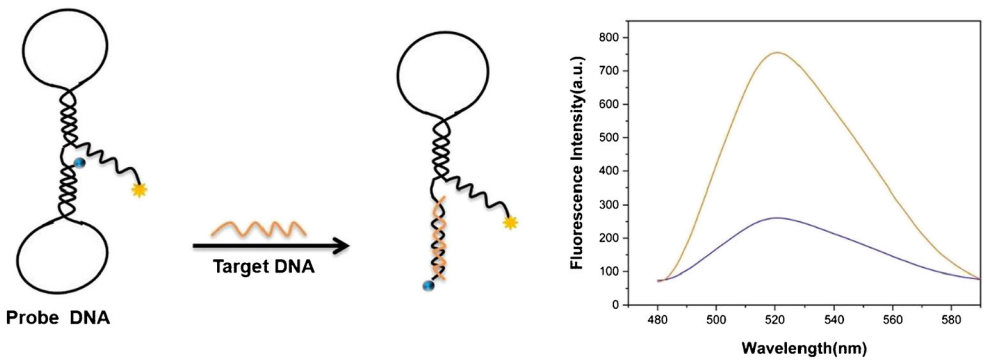

Fig. 3 (Left) Scheme of fluorescence probe conformation behavior; (right) fluorescence emission spectra of a 50-nM fluorescence probe (a). After hybridized with target DNA (b) buffer 10 mM MOPS containing 50 mM $NaNO_3$

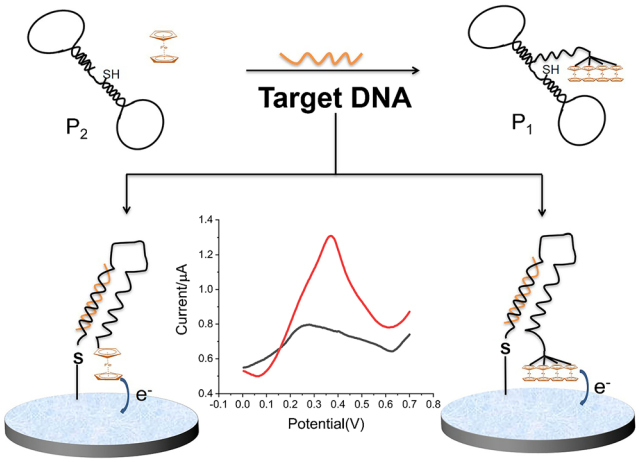

Fig. 4 Hybridization of two different types of probes to target DNA

Electrochemical assay of target DNA

In the experiment, the amount of target DNA was converted into electrochemical signals through homogeneous hybridization and potential-assisted Au-S deposition, which verified the linear range of target DNA. Therefore, the peak current of target DNA with various concentrations was detected using DPV, where three groups of parallel experiments were conducted for every DNA concentration. The average values are shown in Fig. 5(A). Before the experiment, the static voltage was set at −0.2 and tetraferrocene was allowed to stand for 1 min. The currents of DPV were gradually increased with the increasing concentrations of target DNA, indicating that signal responses relied highly on the target concentrations. The current was linearly logarithmically positively correlated with the target DNA concentration within this range from 0.18 to 1800 pM, where the relationship was expressed as $y = 0.2095 \log x + 3.3497$. The value of R^2 (correlation coefficient) was 0.9974 (Fig. 5(B)). The electrochemical sensor exhibited a good linear correlation range from 0.18 to 1800 pM with a detection limit of 0.14 pM, corresponding to the signal of blank plus 3 times the standard deviation (SD). It means that high sensitivity can enlarge the signal.

DPV was also implemented to investigate the sensitivity of the electrochemical sensor with tetraferrocene as signal molecule under conditions of phosphate-buffered saline (PBS) buffer containing probe and different target DNA concentrations. As shown in Fig. 6, in the voltage range of −0.1 to 0.8 V, the non-complementary DNA promoted a small and unavoidable current signal (signal a) when target DNA was not present. The three-base mismatched DNA sequence produced signal (c). DPV signal (d) was generated by the single-base mismatch DNA sequences approximately 1/3 of the DPV signal (d) produced by the fully complementary DNA sequence. Selective hybridization between hairpin probe and target DNA sequences was partially due to inherent recognition ability of DNA bases. Even if the probe hybridized with the mismatched target, its hairpin structure would be partially opened, and when this "hybridization" contacted the electrode, there was still an obvious spatial effect.

This work provided a simple, immobilization-free electrochemical DNA sensor for amplification using tetraferrocene as a signal marker to accomplish a relatively sensitive and efficient test result. In comparison with other DNA amplification methods that employ electrochemically active substances (Table 1), tetraferrocene as a signal amplification marker was directly utilized avoiding additional reagents such as

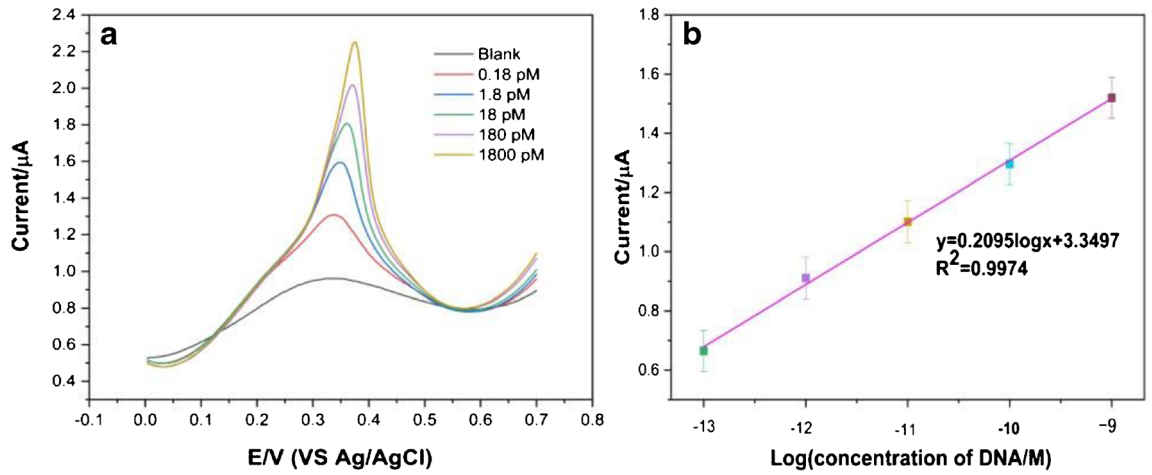

Fig. 5 (A) DPV curve of the probe at different target DNA concentrations (0 M, 0.18 pM, 1.8 pM, 18 pM, 180 pM, and 1800 pM). (B) The corresponding calibration plots

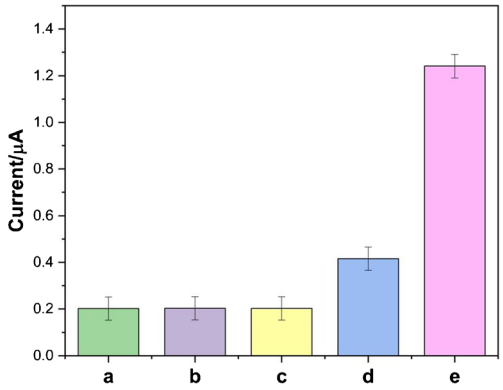

Fig. 6 Comparison of DPV signals of tetraferrocene between probe at − 0.1 to 0.8 V and hybrid buffer without deoxyribonucleic acid sequence (a), 180-pM non-complementary DNA (b), 180-pM three-base mismatched DNA (c), 180-pM single-base mismatched DNA (d), and 180 M target DNA (e)

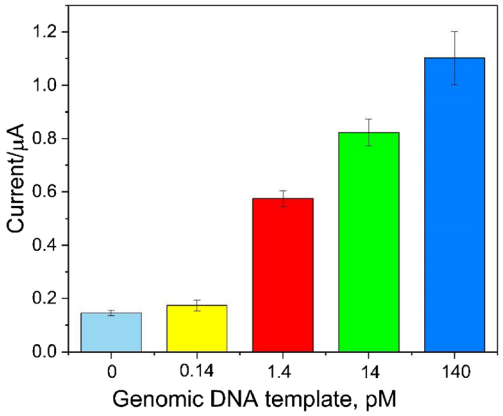

Fig. 7 Analysis result of the PCR product from *Cordyceps sinensis* genomic DNA by using the electrochemical bonding–based DNA sensor

nanometers [33], enzymes [34–36], and multiple probes [22, 35, 36]. The one-time hybridization experiment reduced the incubation time and made the operation easier.

Analytical application in real samples

We investigated the practicability of our biosensor by detecting the polymerase chain reaction (PCR) product, and 158-bp *Cordyceps sinensis* gene was selected as template DNA. As shown in Fig. 7, we found that the current signal increased along with the increase of the template concentration. Finally, as few as 1.4-pM DNA template PCR product was successfully detected in the PCR environment (see Supporting Information (SI) for details), which indicated that the electrochemical DNA sensor was a promising method for the rapid detection of PCR product.

Conclusion

A non-modification electrochemical DNA biosensor was completed, which allowed the hybridization between DNA probe and target DNA to occur in homogeneous solution. Based on the electrochemical bonding method, the sensor can obtain the signal of the specific target sequence simply, quickly, and efficiently without pre-modifying the electrode. In addition, we synthesized a new compound, tetraferrocene, by chemical synthesis to construct probe DNA, which significantly achieved signal amplification. The detection limit as low as 0.14 pM was obtained in the linear range of 0.18–1800 pM. And it is anticipated that the sensor using tetraferrocene as the signal marker can be extended to other simple and real-time detection methods of other electrochemical biosensors.

Table 1 Comparison of our strategy with other methods for electrochemical sensors

Signal amplification	Signal marker	Electrode- modified material	Number of probes	Electrode modification time	Linear range	Detection limit	Reference
Nicking endonuclease assisted Target recycling amplification	Methylene blue	immobilization-free	1	60 min	1 to 10 pM	0.35 pM	34
Exo III-assisted recycling amplification	Methylene blue; Ferrocene	Graphene-modified electrode	2	110 min	1 to 100 nM	0.22 pM.	36
Exo III-assisted recycling amplification	Methylene blue	Immobilization-free	1	0	1 ~ 500 nM	0.43 nM	35
Gold nanoparticles for signal amplification	Methylene blue	Triplex- forming oligonucleotides	1	2 h	0	3 nM	33
Hybridization chain reaction (HCR) signal amplification	Methylene blue indicator	Immobilization-free	2	5.5 h	1 to 800 pM.	1 pM	23
Signal-off based on triple-helix conformation	Methylene blue	Immobilization-free	2	18h	0.5 to 80 pM	0.12 pM	16
Tetraferrocene	Tetraferrocene	Immobilization-free	1	0	0.18 to 1800 pM	0.14 pM	This work

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Liu YH, Deng HH, Li HN, Shi TF, Peng HP, Liu AL, Chen W, Hong GL (2018) A DNA electrochemical biosensor based on homogeneous hybridization for the determination of Cryptococcus neoformans. J Electroanal Chem 827:27–33. https://doi.org/10.1016/j.jelechem.2018.09.001
2. Zhang FT, Cai LY, Zhou YL, Zhang XX (2016) Immobilization-free DNA-based homogeneous electrochemical biosensors. TrAC Trends Anal Chem 85:17–32. https://doi.org/10.1016/j.trac.2016.08.012
3. Li X, Dou BT, Yuan R, Xiang Y (2019) Mismatched catalytic hairpin assembly and ratiometric strategy for highly sensitive electrochemical detection of microrna from tumor cells. Sensors Actuators B Chem 286:191–197. https://doi.org/10.1016/j.snb.2019.01.152
4. Park C, Park H, Lee HJ, Lee HS, Park KH, Choi CH, Na S (2019) Double amplified colorimetric detection of DNA using gold nanoparticles, enzymes and a catalytic hairpin assembly. Microchim Acta 186(1):34 https://xs.scihub.ltd/10.1007/s00604-018-3154-2
5. Lan L, Yao Y, Ping J, Ying Y (2019) Ultrathin transition-metal dichalcogenide nanosheet-based colorimetric sensor for sensitive and label-free detection of DNA. Sensors Actuators B Chem 290:565–572. https://doi.org/10.1016/j.snb.2019.04.016
6. Mandal TK, Parvin N, Mishra K, Mohandoss S, Lee YR (2019) Sensitive and selective fluorometric determination of DNA by using layered hexagonal nanosheets of a covalent organic framework prepared from p-phenylenediamine and benzene-1, 3, 5-tricarboxaldehyde. Microchim Acta 186(12):833 https://xs.scihub.ltd/10.1007/s00604-019-3944-1
7. Dadmehr M, Karimi MA, Korouzhdehi B (2019) A signal-on fluorescence based biosensing platform for highly sensitive detection of DNA methyltransferase enzyme activity and inhibition Spectrochim Acta A 117731. https://doi.org/10.1016/j.saa.2019.117731
8. Yang X, Liu Q, Wen D, Gao M, Zhang D, Jin Q, Zhang J (2019) Ultrasensitive fluorescence detection of sequence-specific DNA via labeling hairpin DNA probes for fluorescein o-acrylate polymers. Anal Chim Acta 1088:144–149. https://doi.org/10.1016/j.aca.2019.08.060
9. Karimi A, Husain SW, Hosseini M, Azar PA, Ganjali MR (2018) Rapid and sensitive detection of hydrogen peroxide in milk by enzyme-free electrochemiluminescence sensor based on a polypyrrole-cerium oxide nanocomposite. Sensors Actuators B Chem 271:90–96. https://doi.org/10.1016/j.snb.2018.05.066
10. Li Y, Qi H, Peng Y, Gao Q, Zhang C (2008) Electrogenerated chemiluminescence aptamer-based method for the determination of thrombin incorporating quenching of tris (2, 2′-bipyridine) ruthenium by ferrocene. Electrochem Commun 10(9):1322–1325. https://doi.org/10.1016/j.elecom.2008.06.027
11. Huang L, Zhang L, Yang L, Yuan R, Yuan Y (2018) Manganese porphyrin decorated on DNA networks as quencher and mimicking enzyme for construction of ultrasensitive photoelectrochemistry aptasensor. Biosens Bioelectron 104:21–26. https://doi.org/10.1016/j.bios.2017.12.037
12. Miao X, Ling L, Shuai X (none) Sensitive detection of glucose in human serum with oligonucleotide modified gold nanoparticles by using dynamic light scattering technique. Biosens Bioelectron 41:880—883. https://doi.org/10.1016/j.bios.2012.09.015
13. Chen JY, Liu ZJ, Wang XW, Ye CL, Zheng YJ, Peng HP, Zhong GX, Liu AL, Chen W, Lin XH (2019) Ultrasensitive electrochemical biosensor developed by probe lengthening for detection of genomic DNA in human serum. Anal Chem 91(7):4552–4558. https://doi.org/10.1021/acs.analchem.8b05692
14. Wu X, Chai Y, Zhang P, Yuan R (2015) An electrochemical biosensor for sensitive detection of microRNA-155: combining target recycling with cascade catalysis for signal amplification. ACS Appl Mater Interfaces 7(1):713–720. https://doi.org/10.1021/am507059n
15. Hansen JA, Wang J, Kawde AN, Xiang Y, Gothelf KV, Collins G (2006) Quantum-dot/aptamer-based ultrasensitive multi-analyte electrochemical biosensor. J Am Chem Soc 128(7):2228–2229. https://doi.org/10.1021/ja060005h
16. Xiong E, Li Z, Zhang X, Zhou J, Yan X, Liu Y, Chen J (2017) Triple-helix molecular switch electrochemical ratiometric biosensor for ultrasensitive detection of nucleic acids. Anal Chem 89(17):8830–8835. https://doi.org/10.1021/acs.analchem.7b01251
17. Hong N, Cheng L, Wei B, Chen C, He LL, Kong D, Ceng J, Cui HF, Fan H (2017) An electrochemical DNA sensor without electrode pre-modification. Biosens Bioelectron 91:110–114. https://doi.org/10.1016/j.bios.2016.10.008
18. Rashid JIA, Yusof NA (2017) The strategies of DNA immobilization and hybridization detection mechanism in the construction of electrochemical DNA sensor: a review. Sensing and Bio-Sensing Research 16:19–31. https://doi.org/10.1016/j.sbsr.2017.09.001
19. Yang Z, d'Auriac MA, Goggins S, Kasprzyk-Hordern B, Thomas KV, Frost CG, Estrela P (2015) A novel DNA biosensor using a ferrocenyl intercalator applied to the potential detection of human population biomarkers in wastewater. Environ Sci Technol 49(9):5609–5617. https://doi.org/10.1021/acs.est.5b00637
20. Sha Y, Zhang Y, Xu E, Wang Z, Zhu T, Craig SL, Tang C (2018) Quantitative and mechanistic mechanochemistry in ferrocene dissociation. ACS Macro Lett 7(10):1174–1179. https://doi.org/10.1021/acsmacrolett.8b00625
21. Li W, Liu X, Hou T, Li H, Li F (2015) Ultrasensitive homogeneous electrochemical strategy for DNA methyltransferase activity assay based on autonomous exonuclease III-assisted isothermal cycling signal amplification. Biosens Bioelectron 70:304–309. https://doi.org/10.1016/j.bios.2015.03.060
22. Hou T, Li W, Liu X, Li F (2015) Label-free and enzyme-free homogeneous electrochemical biosensing strategy based on hybridization chain reaction: a facile, sensitive, and highly specific microRNA assay. Anal Chem 87(22):11368–11374. https://doi.org/10.1021/acs.analchem.5b02790
23. Balaji Viswanath K, Krithiga N, Jayachitra A, Sheik Mideen AK, Amali AJ, Vasantha VS (2018) Enzyme-free multiplex detection of Pseudomonas aeruginosa and Aeromonas hydrophila with ferrocene and thionine-labeled antibodies using ZIF-8/Au NPs as a platform. ACS Omega 3(12):17010–17022. https://doi.org/10.1021/acsomega.8b02183
24. Rasheed PA, Sandhyarani N (2017) Electrochemical DNA sensors based on the use of gold nanoparticles: a review on recent developments. Microchim Acta 184(4):981–1000 https://xs.scihub.ltd/10.1007/s00604-017-2143-1
25. Hua X, Zhou X, Guo S, Zheng T, Yuan R, Xu W (2019) Determination of Alzheimer biomarker DNA by using an electrode modified with in-situ precipitated molybdophosphate catalyzed by alkaline phosphatase-encapsulated DNA hydrogel and target recycling amplification. Microchim Acta 186(3):158 https://xs.scihub.ltd/10.1007/s00604-019-3283-2

26. Yan T, Zhu L, Ju H, Lei J (2018) DNA-walker-induced allosteric switch for tandem signal amplification with palladium nanoparticles/metal-organic framework tags in electrochemical biosensing. Anal Chem 90(24):14493–14499. https://doi.org/10.1021/acs.analchem.8b04338
27. Cui L, Wu J, Li J, Ju H (2015) Electrochemical sensor for lead cation sensitized with a DNA functionalized porphyrinic metal–organic framework. Anal Chem 87(20):10635–10641. https://doi.org/10.1021/acs.analchem.5b03287
28. Kou B, Chai Y, Yuan Y, Yuan R (2018) Dynamical regulation of enzyme cascade amplification by a regenerated DNA nanotweezer for ultrasensitive electrochemical DNA detection. Anal Chem 90(18):10701–10706. https://doi.org/10.1021/acs.analchem.8b00477
29. Yang S, You M, Yang L, Zhang F, Wang Q, He P (2016) A recyclable electrochemical sensing platform for breast cancer diagnosis based on homogeneous DNA hybridization and host-guest interaction between cucurbit [7] uril and ferrocene-nanosphere with signal amplification. J Electroanal Chem 783:161–166. https://doi.org/10.1016/j.jelechem.2016.11.027
30. Van Staveren DR, Metzler-Nolte N (2004) Bioorganometallic chemistry of ferrocene. Chem Rev 104(12):5931–5986.2. https://doi.org/10.1021/cr0101510
31. Zhu J, Gan H, Wu J, Ju H (2018) Molecular machine powered surface programmatic chain reaction for highly sensitive electrochemical detection of protein. Anal Chem 90(8):5503–5508. https://doi.org/10.1021/acs.analchem.8b01217
32. Deng C, Pi X, Qian P, Chen X, Wu W, Xiang J (2016) High-performance ratiometric electrochemical method based on the combination of signal probe and inner reference probe in one hairpin-structured DNA. Anal Chem 89(1):966–973. https://doi.org/10.1021/acs.analchem.6b04209
33. Cinti S, Proietti E, Casotto F, Moscone D, Arduini F (2018) Based strips for the electrochemical detection of single and double stranded DNA. Anal Chem 90(22):13680–13686. https://doi.org/10.1021/acs.analchem.8b04052
34. Tan Y, Wei X, Zhao M, Qiu B, Guo L, Lin Z, Yang HH (2015) Ultraselective homogeneous electrochemical biosensor for DNA species related to oral cancer based on nicking endonuclease assisted target recycling amplification. Anal Chem 87(18):9204–9208. https://doi.org/10.1021/acs.analchem.5b01470
35. Fu C, Liu C, Li Y, Guo Y, Luo F, Wang P, Lin Z (2016) Homogeneous electrochemical biosensor for melamine based on DNA triplex structure and exonuclease III-assisted recycling amplification. Anal Chem 88(20):10176–10182 https://pubs.acs.org/doi/abs/10.1021/acs.analchem.6b02753
36. Liu Y, Ge Z, Chen M, He H, Zhang X, Wang S (2019) Ratiometric electrochemical biosensor based on Exo III-assisted recycling amplification for the detection of CAG trinucleotide repeats. Biosens Bioelectron 142:111537. https://doi.org/10.1016/j.bios.2019.111537