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ORIGINAL PAPER

A sensitive electrochemiluminescence DNA biosensor based on the signal amplification of ExoIII enzyme-assisted hybridization chain reaction combined with nanoparticle-loaded multiple probes

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Abstract

An electrochemiluminescence (ECL) DNA biosensor based on ExoIII exonuclease assistance and hybridization chain reaction (HCR) amplification technology has been constructed. ExoIII exonuclease and triple-helix DNA molecular switch are used in detecting a target in circulation. By combining HCR with AuNPs@DNA, a novel signal probe is built, which enables multiple signal amplification and the high-sensitive detection of transgenic rice BT63 DNA. The Fe_3O_4@Au solution is added to a magneto-controlled glassy carbon electrode, and sulfhydryl-modified capture DNA (CP) is immobilized on Fe_3O_4@Au through the Au–S bond. Mercaptoethanol is added to close sites and prevent the nonspecific adsorption of CP on the magnetron glassy carbon electrode. A target DNA is added to a constructed triple-helix DNA molecular centrifuge tube for reaction. Owing to base complementation and the reversible switching of the triple-helix DNA molecular state, the target DNA turns on the triple-helix DNA molecular switch and hybridizes with a long-strand recognition probe (RP) to form a double-stranded DNA (dsDNA). Exonuclease ExoIII is added to specifically recognize and cut the dsDNA and to release the target DNA. The target DNA strand then circulates back completely to open the multiple triple-helix DNA molecular switch, releasing a large number of signal transduction probes (STP). To hybridize with CP, a large amount of STP is added to the electrode. Finally, a AuNPs@DNA signal probe is added to hybridize with STP. H1 and H2 probes are added for the hybridization chain reaction and the indefinite extension of the primer strand on the probe. Then, tris-(bipyridyl)ruthenium(II) is added for ECL signal detection with PBS-tri-n-propylamine as the base solution. In the concentration range 1.0×10^{-16} to 1.0×10^{-8} mol/L of the target DNA, good linear relationship was achieved with the corresponding ECL signal. The detection limit is 3.6×10^{-17} mol/L. The spiked recovery of the rice samples range from 97.2 to 101.5%. The sensor is highly sensitive and has good selectivity, stability, and reproducibility.

Keywords DNA biosensor · Hybridization chain reaction · Exonuclease · Triple-helix DNA molecular switch · Electrochemiluminescence · Signal amplification

Introduction

Rice is one of the food crops with large edible populations and plays an extremely important role in human survival and development. Insect pests are currently considered the main reason that restricts farmers from obtaining high and stable rice yields. The genetically modified rice BT63 has strong resistance against insects and thus does not require the extensive use of pesticides [1]. To date, using genetic engineering technology in obtaining genetically modified insect-resistant rice is one of the most effective methods for preventing the exposure of rice to pests [2]. However, the safety of BT63 and its food products needs further verification mainly through the detection of insect-resistant genes and their contents [3, 4]. Therefore, reliable detection methods for detecting BT63 are urgently needed.

Scientists are currently working on novel genetically modified techniques for target detection at the protein, RNA, and DNA levels [5]. The main detection methods include PCR detection [6], loop-mediated isothermal amplification technology [7], gene chip [8], RT-PCR detection [9], enzyme-linked

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immunosorbent assay [10], and test strip detection method [11]. Unfortunately, these methods have the following disadvantages: low sensitivity; demanding requirements, such as high-cost reagents and large number of instruments and primers; and complicated operation [12]. Electrochemical biosensors are simple to operate, have high sensitivity, and are low in cost and have thus attracted interest in DNA detection [13–16].

Traditional electrochemical biosensors constructed using single-stranded DNA molecular switches need to be labeled to recognize elements, which interfere with the specificity of detection and thereby reduce stability and alter the loose structures of single-stranded DNA molecules [17]. The reversible switching of molecular switch states and strong background signals is difficult to achieve with electrochemical biosensors constructed from dsDNA molecules [18]. To solve the above problems, a special triple-helix DNA molecular switch (THMS) configuration is established [19, 20], which is based on the principle of the complementary pairing of Watson-Crick and Hoogsteen bases; a DNA strand is inserted to the major groove of a dsDNA structure through hydrogen bonding, and then they are twisted together in a parallel or divergent fashion to form a triple-helix DNA molecular switch that has the advantages of high sensitivity, specificity, low background signal, and reversible state switching. Therefore, this configuration is widely used in the field of electrochemical biosensing research.

The detection sensitivity of electrochemical DNA biosensors can be improved by amplifying target objects and enhancing detection signals; the signal amplification technology, which facilitates target amplification, is the most commonly used method for improving sensitivity. As one of the DNA-mediated self-assembly amplification methods, hybrid chain reaction (HCR) [21–27] provides a highly ordered DNA double helix by triggering DNA polymerization in the presence of initiators or target molecules, and signal molecules can be precisely controlled and attached to a DNA double helix with a high density, which increases the final efficiency of nucleic acid amplification [28]. A fast and efficient amplification platform, HCR, is a simple, enzyme-free, and isothermal amplification technology [29]. However, combining electrochemical detection with HCR-mediated nucleic acid amplification for the high-sensitivity and high-precision detection of genetically modified crop DNA remains challenging.

This study presents a strategy that enables ExoIII exonuclease and triple-helix DNA molecular switch to facilitate the detection of targets in circulation. An AuNPs@(DNA-HCR) signal probe was constructed from AuNPs@DNA through HCR, and a novel and highly sensitive electrochemical DNA biosensor was prepared and used in detecting BT63. First, Fe_3O_4@Au solution was added to a magneto-controlled glassy carbon electrode, and sulfhydryl-modified capture DNA (CP) was immobilized on Fe_3O_4@Au through the Au–S bond. Then, a THMS was constructed, and a target DNA was added. A triple-helix molecule was unlocked through complementary hybridization, and ExoIII exonuclease was added to cut dsDNA. The target DNA and a large number of signal transduction probes (STPs) were released for double recycling. A novel type of signal probe was constructed through HCR to amplify multiple signals. Finally, tris-(bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) was embedded in the extended dsDNA to detect ECL signals. This sensor greatly expands detection signals and enhances the sensitivity of detection and is thus of great significance to food safety.

Experiment

Instruments and reagents

ECL signals were detected with an MPI-E electrochemiluminescence analyzer (Xi'an Remex Analytical Instrument Co., Ltd., Xi'an, China). Electrochemical measurements were performed on a PGSTAT128N Autolab electrochemical workstation (Swiss Metrohm Co., Ltd.) or CHI660D electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd.). A common three-electrode system (Tianjin Yingke United Technology Co., Ltd.) was used for the experiment. It was equipped with a modified magnetically controlled glass carbon electrode (d = 3 mm) as the working electrode, a platinum wire as the auxiliary electrode and Ag/AgCl (saturated KCl solution) as the reference electrode.

Tris (2-carboxyethyl) phosphine (TCEP), mercaptoethanol (MCH), and tris(2,2′-bipyridine)ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2{\cdot}6H_2O$) were purchased from Aladdin Company (Shanghai, China); exonuclease III (ExoIII) 5 U, 6× loading buffer, TE buffer (pH 8.0), and the base sequences were purchased from Shanghai Bioengineering Engineering Company. The base sequences were as follows:

Target DNA BT63: 5′-CGGACGAGTGCTGGGGCAGAT-3′
Recognition probe (RP): 5′-TCCCTTTATCTGCCCCAGCACTCGTCCGTTTCCCT-3′
Signal transduction probe (STP): 5′-AAACCCAGGGAAATTCCC-3′
Capture DNA (CP): 5′-HS-GAT GGGAATTTCCCT-3′
Primer DNA 1 (H1): 5′-CTCATGCGACGT-3′
Primer DNA 2 (H2): 5′-CATGAGACGTCG-3′
Probe DNA: 5′-HS-GCGTTTGGG-3′
Assistant DNA: 5′-HS-TCAGGTACGTCG-3′
One-base mismatched sequence: 5′-CGGACGATTGCTGGGGCAGAT-3′
Three-base mismatched sequence: 5′-CGTACGAGTGATGGGGCAGCT-3′
Noncomplementary sequence: 5′-ACGGACCTGTACATTTGCCGC-3′

Preparation of magnetic Fe_3O_4@Au nanoparticles

Magnetic Fe_3O_4@Au nanoparticles were synthesized according to a previous method [30] with slight modifications. First, 0.670 g of $FeSO_4 \cdot 7H_2O$ and 1.140 g of $FeCl_3 \cdot 6H_2O$ were dissolved in double-distilled water and diluted to 250 mL. The solution mixture was then poured into a three-necked flask constantly protected by nitrogen. A 2 mol/L NaOH solution was quickly added to water bath pot preheated to 50 °C to adjust the pH to 10, and nitrogen was continuously applied for 1.5 h. The solution was constantly stirred. The temperature of the water bath pot was raised to 80 °C, and then the pH of the solution was measured every 10 min. The water bath pot was heated for 30 min and cooled slowly to 20 °C. Magnetic separation was then performed with a strong magnet. Double-distilled water was added every 3 minutes, and then the upper liquid was removed until the lower solution had a neutral PH. Double-distilled water was added until a 5 mg/L Fe_3O_4 solution was obtained to prevent the agglomeration of nanoparticles. The solution was placed in a low-temperature (4 °C) environment for subsequent use.

Approximately 0.229 g of sodium citrate was dissolved in 100 mL of double-distilled water and poured into the flask. The solution was heated in an oil bath and stirred continuously until the temperature reached 99 °C. Then, 1 mL of 5 mg/L Fe_3O_4 solution and 1 mL of 1 g/mL $HAuCl_4$ solution were added under continuous heat and stirring for 15 min. After heating, the solution was stirred until a wine red liquid was obtained, then slowly cooled to 20 °C, magnetically separated with a super strong magnet, and finally washed with double-distilled water to neutrality. The solution was diluted to a volume of 20 mL with double-distilled water for the production of a 0.25 mg/L Fe_3O_4@Au magnetic nanoparticles solution, which was stored under a low temperature (4 °C) for subsequent use.

Preparation of gold nanoparticles

Gold nanoparticles were prepared according to the method described in the literature [31]. Glassware was washed with freshly with aqua regia before the test, washed repeatedly with double-distilled water, and dried with N_2 for subsequent use. Then, 1 mL of $HAuCl_4 \cdot 3H_2O$ (1%) solution and 99 mL of double-distilled water were added to a 250-mL three-necked flask. Under magnetic stirring, 3 mL of sodium citrate buffer (1%) was added and reacted at 120 °C for 1.5 h. After the color changed to wine red, the solution was cooled to room temperature and stored at 4 °C for subsequent use.

Preparation of AuNPs@DNA signal probe solution

Exactly 10 μL of 100 μmol/L probe DNA, 10 μL of 100 μmol/L assistant DNA, and 10 μL of 100 μmol/L TCEP were added to 20 μL of AuNPs solution. TCEP was used to activate the sulfhydryl group at the end of the DNA strand. The solution was completely mixed under ultrasonic conditions and incubated in a constant temperature oven (GZX-GF.101–3-S, Shanghai Yuejin Medical Instruments Factory, Shanghai, China) at 36.8 ± 0.5 °C for 2 h for the production of a AuNPs@DNA reaction solution, which was then stored in a refrigerator at 4 °C for subsequent use.

Pretreatment of magneto-controlled glassy carbon electrode

The magneto-controlled glass carbon electrode was polished with Al_2O_3 powder of 0.1 μm in diameter on the fur and then cleaned in an ultrasonic cleaner with double-distilled water for 10 min. Then, 0.03 μm Al_2O_3 powder was used in painting an "8" shape on the fur for polishing and then washed in the ultrasonic cleaner with double-distilled water for 10 min. Al_2O_3 powder on the surfaces of the magneto-controlled glass carbon electrodes and other specific substances adsorbed were removed. The bare electrode prepared was used as the working electrode. A Ag/AgCl (saturated KCl solution) electrode was used as the reference electrode, and platinum wire electrode was used as the counter electrode. Cyclic voltammetry experiment was then carried out on a CHI660D electrochemical working station. A 0.05 mol/L $[Fe(CN)_6]^{3-/4-}$ working fluid was used (containing 0.1 mol/L KCl). The scanning potential was set at −0.2–0.6 V in advance, and the scanning speed was 0.1 V/s. When a complete and stable redox peak appeared and peak potential difference ΔEp was <0.10 V, a bare magneto-controlled glass carbon electrode (MGCE) that can make good contact with DNA was obtained and then washed with double-distilled water and ethanol for subsequent use.

Preparation of electrochemiluminescence DNA biosensor

This study is based on the triple-helix molecular switch and enzyme-assisted target cycle detection. By combining AuNPs and HCR, a new signal probe was constructed for the amplification of multiple signals and construction of an efficient, rapid, and highly sensitive ECL biosensor that quantitatively and accurately detect BT63 in genetically modified rice. First, 15 μL of 0.25 mg/L Fe_3O_4@Au solution was collected with a pipette and then dropped on the prepared GCE. The solution was heated in an oven (36.8 ± 0.5 °C) for 15 h. Then, 10 μL of 1 μmol/L sulfhydryl-modified capture DNA (CP) and 10 μL of 10 mmol/L TCEP were added, dropped to the electrode for mixing, and incubated for 2 h. TCEP activated the terminal sulfhydryl of the CP, inducing the self-assembly of Fe_3O_4@Au surface by CP through the Au–S covalent bonds. Exactly 10 μL of MCH was added to close the sites and

prevent the nonspecific adsorption of CP on the magneto-controlled glass carbon electrode. The target DNA (15 μL) was added to the constructed triple-helix DNA molecular centrifuge tube with a pipette and reacted for 80 min in an oven (36.8 ± 0.5 °C). Owing to base complementation and the reversible switching of the triple-helix DNA molecule state, the target DNA turned on the triple-helix DNA molecule switch. Approximately 15 μL of 2.0 U/μL ExoIII exonuclease and 10× enzyme buffer were added to cut the dsDNA and release the target DNA. The target DNA strand circulated and turned on multiple triple-helix DNA molecular switches, thereby promoting a cyclic process and releasing a large number of STPs. A large number of STPs hybridized with CP. The AuNPs@DNA (20 μL) signal probe was added for complementary hybridization with STP, and 20 μL of H1 and H2 were added and reacted for 2 h for the hybridization of the DNA on the probe. $Ru(bpy)_3Cl_2$ was added to the modified electrode to react for 2 h, and PBS-tri-n-propylamine was used as the base solution for ECL signal detection. After each step, the obtained modified electrode was washed once with TE buffer and double-distilled water successively, and unconnected substances were washed away before subsequent steps. The principle is shown in Fig. 1.

Electrochemiluminescence detection

ECL signal detection was carried out through cyclic voltammetry (CV). The prepared magnetically controlled glass carbon electrode was used as the working electrode. The electrode was a platinum wire electrode, the reference electrode was a Ag/AgCl (saturated KCl) electrode, and 2 mL of PBS-TPA (0.1 mol/L, pH 7.5, 0.02 mol/L TPA) solution was used as the working fluid. The scan parameters were as follows: scanning speed, 100 mV/s; scanning potential range, 0–1.25 V. The CV and electrochemical impedance spectroscopy (EIS) experiments can be used in determining how electrode surface modification affects probe redox and its reversible/irreversible behavior. The measurement was performed at a scanning speed of 100 mV/s and scanning potential range of −0.2–0.6 V in 0.05 mol/L $[Fe(CN)_6]^{3-/4-}$ solution (containing 0.1 mol/L KCl). An EIS experiment was performed under the same base conditions as CV at a potential of 0.19 V, amplitude of 5 mV, and frequency range of 0.1–100,000 Hz.

Results and discussion

Characterization of transmission electron micrograph (TEM) of the Fe_3O_4@Au magnetic nanoparticles and the particle size of the Au nanoparticles

The magnetic nanoparticle Fe_3O_4@Au was used to immobilize DNA and increase DNA loading capacity. It was also used to facilitate the renewal of the prepared DNA biosensor. The TEM of Fe_3O_4@Au magnetic nanoparticles (Fig. 2a) shows that the nanoparticles are evenly dispersed and have an average particle size of approximately 20 nm. The particle size of Fe_3O_4 is about 13 nm. In addition, the particle size analysis experiment shows that the Au nanoparticles have a particle size of approximately 24 nm (Fig. 2b).

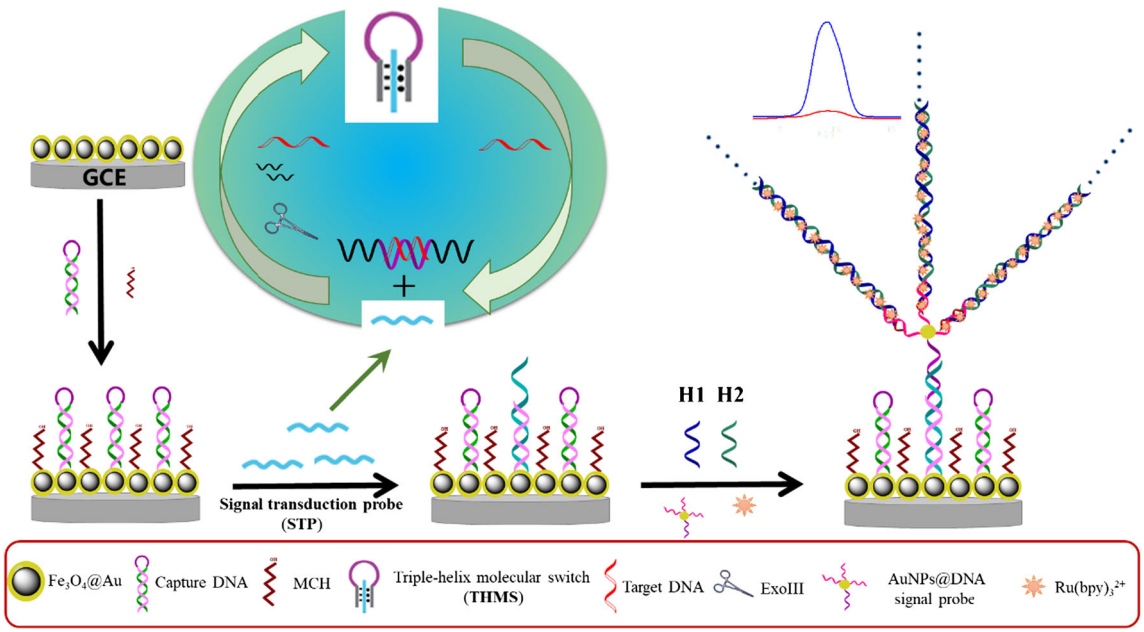

Fig. 1 Schematic diagram of the electrochemical biosensor for the high-sensitivity detection of transgenic rice BT63 based on ExoIII enzyme and hybrid chain reaction (HCR)

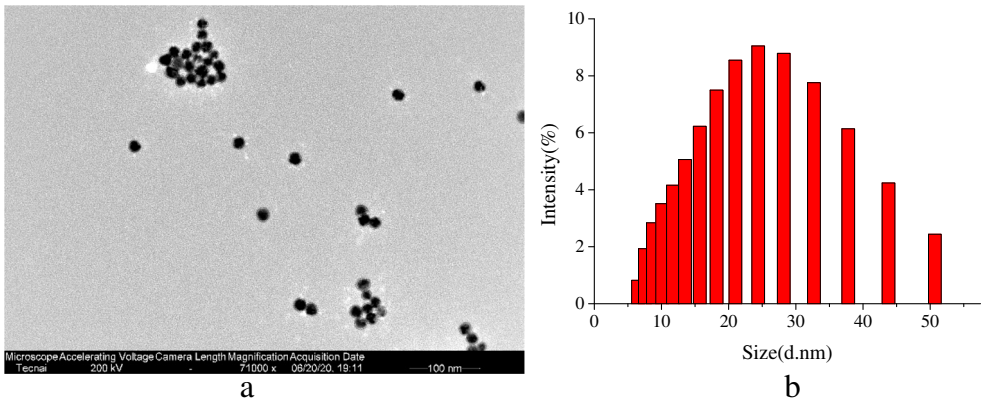

Fig. 2 **a** TEM of the Fe_3O_4@Au magnetic nanoparticles, **b** particle size distribution of the AuNPs

CV and AC characterization of electrode modification process

Figure 3 shows the CV characterization of the sensor preparation. CV is an effective tool for characterizing the electrochemical performance of modified electrodes. Figure 3a shows the bare electrode CV curve where the current signal is the highest. Figure 3b shows Fe_3O_4@Au fixed on the bare electrode, where the number of electrode surface sites and current decrease. Figure 3c indicates that CP self-assembles to the electrode through Au–S bond. Resistance increases and current decreases after the addition of the capture probe CP and TCEP to the modified electrode. As shown in Fig. 3d, after the constructed triple-helix DNA molecular switch reacts with the target DNA and ExoIII specifically recognizes the cut dsDNA, it releases a large number of STPs, which hybridize with CP, electron transfer is blocked, and current decreases. When the AuNPs@DNA signal probe was added, electrode surface resistance increases, and current further decreases (Fig. 3e). After the addition of H1 and H2, the DNA primer strand on the probe undergoes HCR through complementary hybridization (Fig. 3f). Owing to the continuous amplification of the double strand, the electron transfer rate is greatly reduced because of the increase in chain length. Thus, current is obviously reduced. The same conclusions mentioned above were also obtained through the EIS characterization of six identical modified electrodes, as shown in Fig. 4.

Optimization of experimental conditions

Experimental conditions, including the amount of Fe_3O_4@Au, the hybridization time of the target DNA, the amount of exonuclease ExoIII, the incubation time of

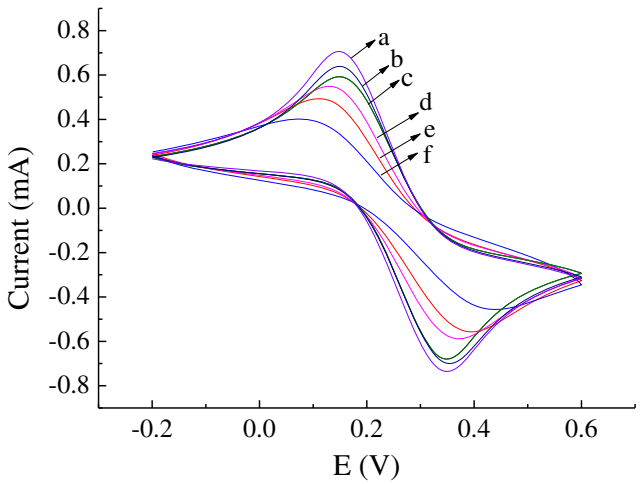

Fig. 3 Cyclic voltammograms of different processes on modified electrode CVs of the different electrodes in 0.05 mol/L $[Fe(CN)_6]^{3-/4-}$: **a** bare electrode; **b** Fe_3O_4@Au; **c** capture DNA (CP)/TCEP; **d** signal transduction probe (STP); **e** AuNPs@DNA signal probe; **f** hybrid chain reaction (HCR) process. Scan rate: 100 mV/s

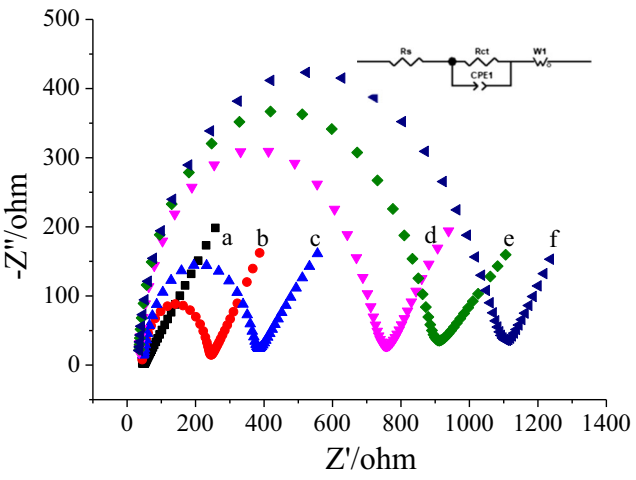

Fig. 4 EIS characterization of different electrodes in 0.05 mol/L $[Fe(CN)_6]^{3-/4-}$: **a** bare electrode; **b** Fe_3O_4@Au; **c** capture DNA (CP)/TCEP; **d** signal transduction probe (STP); **e** AuNPs@DNA signal probe; **f** hybrid chain reaction (HCR) process. Scan rate: 0.1 V/s

Table 1 Comparison of the present method with other reported methods

Detection methods	Linear range (mol/L)	Detection limit (mol/L)	References
Strand displacement isothermal amplification (SDA)	1.0×10^{-15}~1.0×10^{-10}	6.5×10^{-16}	[32]
Polymerase chain reaction (PCR)	6.0×10^{-10}~3.0×10^{-8}	3.0×10^{-11}	[33]
Loop-mediated isothermal amplification (LAMP)	1.0×10^{-11}~1.0×10^{-6}	1.8×10^{-12}	[34]
Ligase chain reaction (LCR)	1.0×10^{-13}~1.5×10^{-7}	1.4×10^{-15}	[35]
Hybridization chain reaction (HCR)	1.0×10^{-16}~1.0×10^{-8}	3.6×10^{-17}	This Work

exonuclease ExoIII, and the time of $Ru(bpy)_3^{2+}$ intercalation into dsDNA, were optimized. The results are shown in Fig. S1–S3. As shown in Fig. S1, ECL signal increases significantly with the amount of Fe_3O_4@Au and target DNA hybridization time. When the dosage of Fe_3O_4@Au is 15 μL and the hybridization time of the target DNA is 80 min, the ECL signal intensity peaks and tends to stabilize. Figure S2 illustrates the effect of the amount of exonuclease ExoIII and the incubation time of exonuclease ExoIII on ECL intensity. The optimal amount of exonuclease ExoIII is 15 μL, and the optimal incubation time is 1.5 h. Finally, the time for $Ru(bpy)_3^{2+}$ to intercalate into dsDNA is optimized. When the time is 2 h, ECL intensity reaches its maximum value (Fig. S3).

Calibration plot

Figure 5 shows the ECL response and calibration curves of the DNA concentrations of different targets under optimal conditions: a–j represent 0, 10^{-16}, 10^{-15}, 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, and 10^{-8} mol/L target DNA. ECL intensity gradually increases with target DNA concentration. As shown in Fig. 5, the target DNA concentration ranges from 1.0×10^{-16} to 1×10^{-8} mol/L, and the ECL intensity detected (I, minus the blank detection value) has a good one-square linear relationship with the logarithmic (logC) of the target DNA concentration, the correlation coefficient r is 0.9982, the detection limit is 3.6×10^{-17} mol/L (S/N = 5), and the linear regression equation is follows: $\Delta I = 206.89 + 1586.3\ \log(C/10^{-16}\ \text{mol/L})$. These results show that the method has a low detection limit and high sensitivity. Compared with other DNA sensors, the sensor exhibits higher sensitivity and has a wider detection range, which is due to the signal amplification of ExoIII

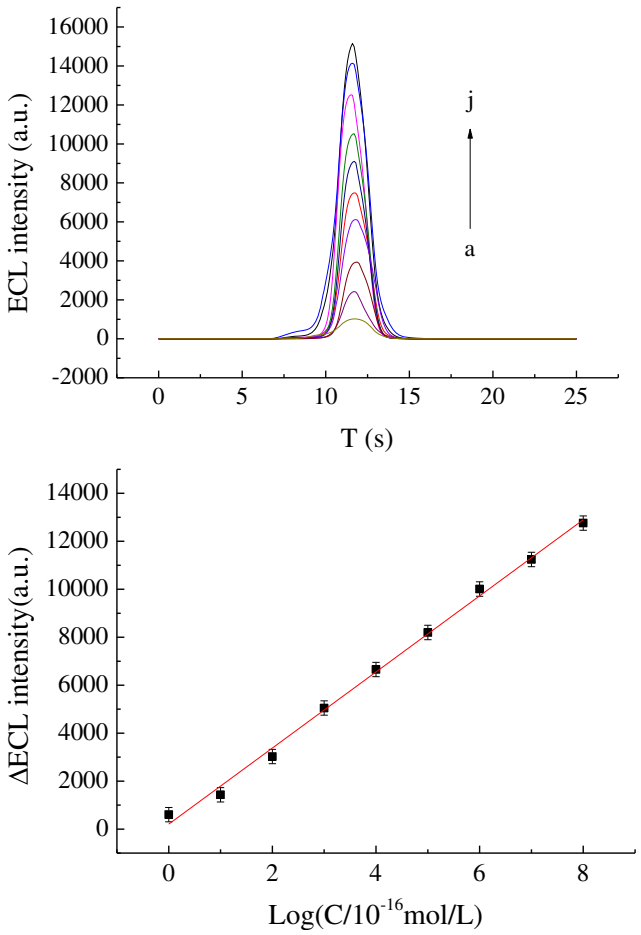

Fig. 5 ECL intensity of the DNA biosensor at different DNA concentrations versus time, and calibration plot from **a** to **j**: 0, 10^{-16}, 10^{-15}, 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, and 10^{-8} mol/L. Scanning speed, 100 mV/s; scanning potential range, 0–1.25 V

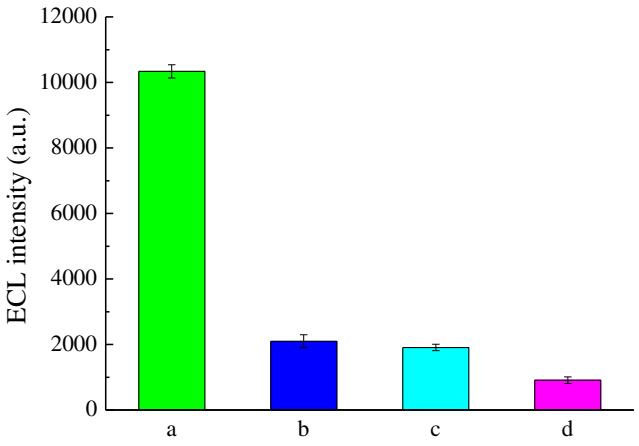

Fig. 6 Selectivity of the fabricated electrochemiluminescence DNA biosensor **a** 1.0×10^{-11} mol/L target DNA, **b** 1.0×10^{-9} mol/L single-base mismatch DNA, **c** 1.0×10^{-9} mol/L three-base mismatch DNA, d 1.0×10^{-9} mol/L noncomplementary strand DNA

Table 2 Analytical results of rice samples

Samples	Found (mol/L)	Added (mol/L)	Total found (mol/L)	RSD (n=5, %)	Recovery (%)
1	Not found	2.00×10^{-13}	2.03×10^{-13}	3.8	101.5
2	Not found	2.00×10^{-14}	1.94×10^{-14}	2.1	97.2
3	Not found	3.00×10^{-15}	2.98×10^{-15}	4.2	99.3

enzyme-assisted HCR combined with nanoparticle-loaded multiple probes (Table 1).

Selectivity of the biosensor

For the verification of the selectivity to the target DNA, the capture probe (CP) was hybridized with 1×10^{-11} mol/L target DNA (a), 1×10^{-9} mol/L single-base mismatched DNA (b), three-base mismatched DNA (c), and noncomplementary DNA (d). As shown in Fig. 6, the ECL signals of the single-base mismatched DNA, three-base mismatched DNA, and noncomplementary DNA detection are weaker than the fully complementary DNA detection signal. The ECL signals generated by the single-base mismatched DNA (b), three-base mismatched DNA (c), and incompletely complementary DNA (d) have minimal effects on the detection of target DNA. Therefore, the electrochemical DNA biosensor prepared in this experiment has excellent selectivity.

Reproducibility and stability of the biosensor

For the testing of the reproducibility and stability of the sensor, the sensor was sealed and stored in a refrigerator at 4 °C for 14 days. The ECL signal is reduced by 9.86% only, showing that the sensor prepared in this experiment has good stability. Under the same conditions, six sensors are prepared to detect 1.0×10^{-12} mol/L target DNA. The relative standard deviation of the six determinations is 3.68%. The results show that the sensor has good reproducibility.

Sample analysis

The practicability of the sensor was verified using rice cakes prepared with non-GMO rice purchased from a supermarket. The samples were then extracted from rice cakes with a rapid plant genomic DNA isolation kit (Sangon Biotech Co., Ltd. Shanghai, China). The purity and concentration of DNA extracted were measured using a Nano-Drop 1000 UV-vis spectrophotometer (NanoDrop, Wilmington, DE, USA). Three DNA standard solutions with known concentrations in the experiment were added to the extracted DNA samples for label recovery experiments. The results are shown in Table 2. The recovery is 97.2–101.5%, and the RSD ($n=5$) is 2.1–4.2%, all lower than 5%, all indicating that the sensor has good practicability.

Conclusions

Based on exonuclease, triple-helix DNA molecular switch, and hybrid chain reaction, a novel ECL DNA biosensor is highly sensitive in detecting genetically modified rice BT63. Exploiting the high-efficiency specificity of exonuclease and easily reversible switching characteristics of the triple-helix DNA molecular switch in target DNA recycling, we constructed a signal probe from AuNPs@DNA through hybridization chain reaction and used it in detecting multiple amplification signals. The amplification technology established in this experiment is of great significance to food safety and has great potential in the high-sensitivity detection of genetically modified products.

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Compliance with ethical standards

Conflict of interest There are no conflicts to declare.

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