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Signal amplification technology based on entropy-driven molecular switch for ultrasensitive electrochemical determination of DNA and *Salmonella typhimurium*

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ABSTRACT

A methodology based on entropy-driven molecule switch signal amplification strategy has been developed for the ultrasensitive detection of DNA. The gold electrode modified with gold nanoparticles was used to immobilize capture hairpin DNA. In the presence of target DNA, the stem of hairpin DNA was opened once the hybridization reaction occurred between the capture DNA and target DNA. With the addition of the link DNA which has more complementary bases with the capture DNA, the link DNA would hybridize with the capture DNA, and the target DNA would be displaced under the entropy-driven. The released target DNA could open another hairpin DNA. Through such a cycle the target DNA could be recycled, and more hairpin DNA was opened and more link DNA would hybridize with the capture DNA. When the electrochemical nanoparticle probe which consisted of the nanoparticles, probe DNA and the electrochemical reagent was added, the link DNA would hybridize with the probe DNA. As a result, the electrochemical response can be amplified and monitored. In this work, the *Salmonella typhimurium* aptamer complementary DNA was chosen as a model target, and *S. typhimurium* was also tested by target induced strand release technology coupling the entropy-driven molecule switch signal amplified strategy. The electrochemical sensor demonstrated excellent sensing performances such as ultra low detection limit (0.3 fmol L^{-1} for DNA and 13 cfu mL^{-1} for *S. typhimurium*) and high specificity, indicating that it is highly promising to provide a sensitive, selective, cost-effective, and convenient approach for DNA and *S. typhimurium* detection.

1. Introduction

Gene-sensors have become tools with great potential for a variety of application: clinical diagnosis, environmental monitoring and biological research. The main available strategies for gene detection involve fluorescent [1,2], radioactive or chemiluminescent markers [3], which usually make use of hazardous compounds and are generally time-consuming. Electrochemical (EC) analysis, which was widely used in many applications, is an attractive candidate sensing technique for gene-sensors owing to its simple and cost-effective characteristics and has been extensively used for the determination of DNA concentrations [4–6]. Gold nanoparticles (AuNPs) were generally used to modify the electrode to immobilize single-stranded nucleic (ss-DNA) for EC detection of DNA by taking advantage of their large surface area and unique chemical properties [7].

Signal amplification has become more and more commonly used in the detection of DNA. It is an effective way to improve the sensitivity of a gene-sensor. So far, enzymes [1,8,9], DNAzymes [10,11], and nanoparticles [12,13] have been employed as amplifying labels for biorecognition events. Moreover, signal amplification techniques such as polymerase chain reaction (PCR) [14–16], rolling circle amplification (RCA), and nicking endonuclease signal amplification have also been reported [17–19]. Among these methods, nicking endonuclease signal amplification received much more attention because of its high amplification efficiency [20–23]. But for the cyclic nicking and polymerization based amplification strategies, they need at least two kinds of enzymes, the

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restriction endonuclease and polymerase, and involve complicated manipulation steps, which limit their broad applications. Therefore, it is important and necessary to develop enzyme-free catalytic cycles for the amplified detection of DNA. Entropy-driven molecule switch method has been proved to be an excellent signal amplification technique with great advantages compared with others: cost-effective, simple testing process and high amplification efficiency [1,24,25]. The molecule switch was completed by autocatalysis driven by the free energy of base pair formation. Autocatalysis could also be completed through entropy-driven hybridization mechanisms. There was no need of enzymes in these processes.

In this work, we described a signal amplification method based on entropy-driven molecule switch for the detection of DNA and *S. typhimurium*. AuNPs were used to immobilize EC reagent on the electrode surface to fabricate EC nanoparticle probe. In the presence of the target DNA, the stem of hairpin DNA was opened once the hybridization reaction occurred between the capture DNA and target DNA. Then the link DNA which has more complementary bases with capture DNA was added, and it would hybridize with the capture DNA and the target DNA would be displaced under the entropy-driven. The released target DNA could open another hairpin DNA. Through such a cycle the target DNA could be recycled, and more hairpin DNA was opened and more link DNA could hybridize with the capture DNA. When the EC nanoparticle probe which consisted of the nanoparticles, probe DNA and the EC reagent was added, the link DNA would hybridize with the probe DNA. As a result, the EC response can be produced and amplified through this process. The experimental results showed that this method was sensitive and selective for the determination of DNA, and it can be used for the detection of *S. typhimurium* in real samples.

2. Experimental

2.1. Chemicals and materials

$HAuCl_4$, Thionin (Th), and sodium citrate ($C_6H_5O_7$) were obtained from Shanghai Chemical Reagent Co. (Shanghai, China); *S. typhimurium* was purchased from the American Type Culture Collection (ATCC); N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxy succinimide (NHS) and imidazole were purchased from Tianjin Bodi Chemical Reagent Co. (Tianjin, China); Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), HS-CH_2CH_2-OH (MCH), Tris(hydroxymethyl)methyl aminomethane (Tris) F were purchased from Guoyao Chemical Company. Carboxyl groups modified magnetic beads (MBs) (1–1.5 μm, 10 mg mL^{-1}) were purchased from Baseline Chromtech Research Centre (Tianjin, China).

All synthetic oligonucleotides were purchased from SBS Genetech Co. Ltd. (Beijing, China). Their sequences were presented as follows:

DNA1: (target DNA) 5′-CAA TAA CTA CCG GGC ATT ACT GGC CTT-3′
DNA2: (capture DNA) 5′-SH-$(CH_2)_6$-AAG GCC AGT AAT GCC CGG TAG TTA TTC CAT CGT GTA AAG AGC ACC CTT GTA CCA ATA ACT ACC GGG CAT-3′
DNA3: (link DNA) 5′-GGT GCT CTT TAC ACG ATG GAA TAA CTA CCG GGC ATC CAT CGT GTA C-3′
DNA4: (probe DNA) 5′-HS-$(CH_2)_6$-TTT TTT ATG CCC GGT AGT TAT TGG TAC AA-3′
DNA5: (aptamer) 5′-NH_2-$(CH_2)_6$-AGT AAT GCC CGG TAG TTA TTC AAA GAT GAG TAG GAA AAG A-3′
One base mismatch DNA: 5′-CAA TAA CT*C* CCG GGC ATT ACT GGC CTT-3′
Two bases mismatch DNA: 5′-CAA TAA CT***C G***CG GGC ATT ACT GGC CTT-3′
Three bases mismatch DNA: 5′-CAA TAA CT***C GA***G GGC ATT ACT GGC CTT-3′

2.2. Apparatus

All the electrochemical experiments were carried out with CHI 660D Electrochemical Analyzer (CH Instruments, Shanghai, China), using a conventional three-electrode system with the gold electrode (GE) or AuNPs modified GE as the working electrode, a platinum wire as the counter electrode, a Ag/AgCl electrode as the reference electrode. The shape and characteristics of AuNPs and AuNPs modified electrodes were examined using a JSM-6700F scanning electron microscope (Hitachi High-Technology Co., Japan) and a JEM-2010 transmission electron microscope (Hitachi High-Technology Co., Japan).

2.3. The synthesis of Au nanoparticles

All glassware was washed with aqua regia firstly, rinsed with distillated water then dried before used. AuNPs was prepared according to the method reported previously with slight modification [26,27]. Briefly, $HAuCl_4$ was dissolved in 100 mL deionized water (0.01%) and then the solution was heated until boiling, and 1.4 mL of 1% sodium citrate solution was added and the mixed solution was stirred for 10 min at the boiling point. The color of the solution gradually changed from faint yellowish to wine red. AuNPs prepared by this method have an average diameter of approximately 20 ± 2 nm as measured by TEM. The prepared 20 nm AuNPs were stored in a brown glass bottle at 4 °C in a refrigerator.

2.4. The preparation of electrochemical nanoparticles probe

Firstly, Thionin (Th) was directly immobilized onto the AuNPs according to reported methods with slight modification [28,29]. 100 μL of 1×10^{-4} mol L^{-1} Th was added into 1 mL AuNPs for 30 min to immobilize Th on their surface. Then 10 μL of 1.0×10^{-5} mol L^{-1} probe DNA, 10 μL of pH 8.2, 50 mM Tris–HCl and 10 μL of 10 mM TCEP were added into a 2 mL tube for 1 h to activate the -SH of probe DNA. Finally, the probe DNA was added into the Th labeled AuNPs and incubated at 37 °C for 16 h with shaking. The product was washed with 1.0 mL of 0.1 mol L^{-1} phosphate buffer solution three times, and resuspended in 1.0 mL phosphate buffer solution and stored at 4 °C for further use.

2.5. The preparation of gold nanoparticles modified electrode

The GE was polished with Al_2O_3 powders and then washed with ethanol and distilled water under ultrasonication, respectively. The electrode was allowed to dry at room temperature. The gold electrode was modified with AuNPs by dropping 10 μL AuNPs onto its surface and then dried in air at room temperature.

2.6. The electrochemical detection of target DNA

The capture DNA and link DNA were pretreated according to the reported method [30] to form the stem-loop structure. The capture DNA and link DNA were firstly denatured at 95 °C for 5 min. Then, they were slowly cooled to room temperature to form stem–loop structures. 10 μL of 1.0×10^{-6} mol L^{-1} capture DNA was dropped onto the AuNPs modified electrode at 4 °C. After 24 h incubation, the electrode was washed with 0.1 mol L^{-1} of phosphate buffer solution for three times, and 10 μL of 1.0×10^{-4} mol L^{-1} MCH was dropped onto the electrode surface to block the active surface area. Then 10 μL of target DNA was dropped onto the electrode surface