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Electrochemical biosensor for DNA damage detection based on exonuclease III digestions

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ABSTRACT

Detection of DNA damage is significant to evaluate genotoxicity of new chemicals in the early stages of its development. In this work, we described an electrochemical biosensor for simple, rapid and sensitive detection of specific sequences of DNA. Ferrocene (Fc)-labeled probe DNA first hybridized with capture DNA 1 to form double strands in the hybridization solution. In the presence of target DNA, the Fc-labeled probe DNA will be released by the digestions of exonuclease III and further hybridize with the capture DNA 2 immobilized on a gold electrode, producing strong electrochemical signals. On the other hand, Exonuclease III (Exo III) leaded to recycling and reuse of the target DNA, which also resulted in signal amplification. The present method can discriminate single base mismatched DNA from complementary DNA and has been successfully applied to detect DNA damage induced by chemical reagents and UV radiation.

1. Introduction

DNA is the most important genetic material of living beings. However, many chemical reagents or physical radiation in our living environment induce damage to DNA. So exposure to the dangerous compounds can increase the chance of getting lesion [1]. The development of sensitive, rapid and inexpensive analytical methods to detect DNA damage is of significance.

Various methods have been developed to detect DNA. It has been proved that signal amplification is an effective way to improve the detection sensitivity and has been extensively used in sensors construction. Many signal amplification methods using tags such as enzymes [2–9], conjugated polymer [10,11], nanoparticles [12–15], and atom transfer radical polymerization [16–18] have been reported.

Xu's group utilized klenow polymerase to trigger polymerization reaction to detect single-nucleotide polymorphisms, the designed triple-stem probe allowed circular hybridization, polymerization reaction and displacement. This process enhanced the electrogenerated chemiluminescence signal and lowered the detection limit to 35 aM [2]. Ju's group took advantage of nicking endonuclease and DNA polymerase to detect nucleic acids. The ultrasensitive protocol is designed by using quantum dots as signal tag based on the enhanced hybridization process and rolling circle amplification, which reduced the limit of detection to 11 aM [3]. Recently, Willner's group used Exo III as a biocatalyst to trigger the recycle of target. In that paper, the binding of the target DNA complex to the functionalized quantum dots leads to the Exo III stimulated recycling of the target DNA [4]. Plaxco's group also used Exo III to design an amplified DNA detection scheme by employing a stem-loop DNA molecular beacon as signal probe. When target is present, the stem-loop structure is opened and forms a double-stranded structure with a blunt 3′terminus. Exo III catalyzes the stepwise removal of mononucleotides from this terminus, liberating the fluorophore before ultimately releasing the target. The released target is circulated, producing many fluorescent molecular beacon fragments. Thus, the amplification leads to a 4-fold increase in the final fluorescence [5]. Yuan's and Hsing's group also used Exo III to recycle and reuse of the target DNA for signal amplification [6,7].

Among the methods reported, electrochemical method has certain advantages because of its high sensitivity, low cost, and excellent compatibility with miniaturization technologies. Compared with other tags [19–21], ferrocene (Fc) tag has attracted great attentions due to its fast electron transfer rate and synthetic versatility [22–24]. Here, we proposed a rapid, simple and sensitive electrochemical sensor for specific DNA detection based on Exo III digestions by using Fc as probes. The proposed method can be used

Abbreviations: Fc, ferrocene; Exo III, Exonuclease III; QDs, quantum dots; UV, ultra violet; SO, Styrene Oxide; SA, sodium arsenite; EIS, electrochemical impedance spectroscopy; DPV, differential pulse voltammetry; PBS, phosphate buffer solution.

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to quantitatively detect specific sequences of DNA from 2.0 pM to 200 nM. The linear correlation coefficient was 0.997 and the detection limit was 0.12 pM. On the other hand, the method has good specificity to discriminate one base mismatched DNA from complementary DNA. DNA damage induced by chemical reagents such as SO, SA, Cu(II)/H_2O_2 and UV radiation have been successfully detected by the method.

2. Experimental

2.1. Reagents

The DNAs used in this paper were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Their sequences are shown in Table 1. The primitive sample was centrifuged at 5000 rpm for 5 min and then dissolved with 100 mM pH 7.4 PBS. Then the solution was deposited at 0–4 °C for storage. Exo III was purchased from Takara Biotechnology Co., Ltd. (Dalian, China).

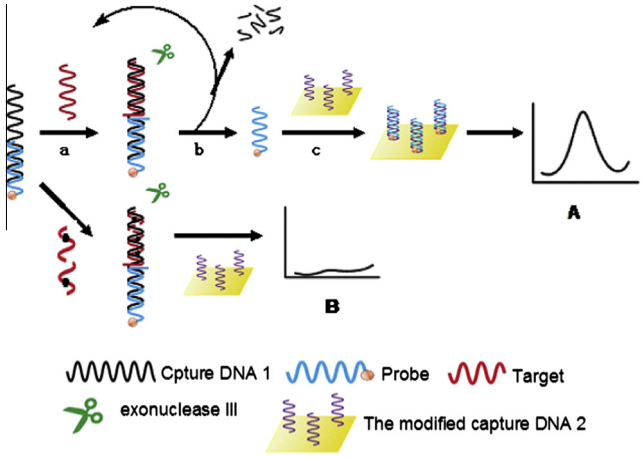
2.2. Apparatus

The electrochemical experiments were performed with a model CHI 750C electrochemical workstation (Shanghai Chenhua Equipments, China). The three-electrode system was consisted of a KCl saturated calomel electrode as the reference, a platinum electrode as the counter electrode, and a gold electrode (2 mm diameter) as the working electrode. The instrument used to heat the sample was DF-101S heat-gathering magnetic heating stirrer (Gongyi Kerui Instrument Co., Ltd.).

2.3. Procedures

2.3.1. Scheme for detection of DNA

The scheme for detection of specific sequences of DNA is shown in Scheme 1. Capture DNA 1 was complementary to the probe DNA at one-half-segment and the target DNA at another half-segment. The capture DNA 1 hybridized with probe DNA firstly at the ratio of 1:1. First, in the presence of target DNA, stable sandwich hybridization was formed, which has a protruding 3′terminus at probe DNA and a blunt 3′terminus at capture DNA 1 (a). Then, Exo III gradually removed mononucleotides from the blunt end of capture DNA 1, releasing the target DNA and Fc-labeled probe DNA (b). Subsequently, the released probe DNA was captured by the capture DNA 2 modified on the gold electrode and produced a redox current (c). It was important that the released target DNA recycled to hybridize with capture DNA 1/probe DNA to trigger a new degraded process, leading to signal amplification (A). In the presence of damaged target DNA, the stable duplex complex cannot be formed, as a result, Exo III cannot degrade the capture DNA 1, and release target and probe DNA efficiently. Thus, electrochemical signal decreased sharply (B). From these different electrochemical signals, the biosensor could be used to distinguish damaged DNA from intact DNA.



Scheme 1. Strategy for detection of DNA damage.

2.3.2. Immobilization of capture DNA 2 on a gold electrode

Gold electrodes (99.99% polycrystalline, 2 mm diameter, CH Instrument Inc.) were treated with Piranha Solution (H_2O_2/H_2SO_4 1:3 in volume), polished on microcloth (Buehler) with 0.3 μm γ-alumina suspension (CH Instrument Inc.) for 2 min, and rinsed with ultrapure water and ethanol followed by drying under nitrogen flow. The pretreated gold electrode was immersed in 100 mM pH 7.4 PBS containing 1 μM capture DNA 2 and 1 M NaCl for 12 h at room temperature. Physically adsorbed DNA was removed by rinsing the electrode. After that, the electrode was immersed in 100 mM pH 7.4 PBS for another 20 min at 50 °C to orient the modified capture DNA 2. Finally, the modified electrode was dried under nitrogen flow [25].

2.3.3. DNA hybridization and digestion

1 μM capture DNA 1 and probe DNA was incubated for 2 h in 100 mM pH 7.4 PBS containing 0.3 M NaCl at 50 °C. When the temperature decreased to 37 °C, Exo III (200 U) and target DNA were added into the solution for incubation for 2 h [26]. Digestions were stopped by heating up the solution to 50 °C for 20 min.

2.3.4. Electrochemical detection of target DNA

The gold electrode immobilized with capture DNA 2 was immersed in the above digestion solutions at 50 °C for 2 h. The electrochemical measurements were performed in 100 mM pH 7.4 PBS containing 0.1 M $KClO_4$ at room temperature [27]. The differential pulse voltammetry (DPV) curves were background-subtracted using ORIGIN 8.5 (Micirocal Software, Northampton, MA) through extrapolation to the baseline in the regions far from the peaks. The electrochemical impedance spectroscopy (EIS) experiments were measured in 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl with the frequency range from 1 Hz to 10 kHz.

Table 1
Sequences of DNAs used in this work.

Oligonucleotides	Sequence (5′–3′)
Target	CAA GAC CAC CAC TTC GAA ACC
One base mismatched DNA	CAA GA<u>C</u> CAC CAC TTC GAA ACC
Three bases mismatched DNA	CAA GA<u>C</u> <u>GT</u>C CAC TTC GAA ACC
Capture DNA 1	CTA TTA AAC ATG CCA CTC GCA ATG AAG TGG TGG TCT TG
Capture DNA 2	SH-GGC CTA TTA AAC ATG GGG A
Probe DNA	ACC GCT TAA TTG CGA GTG GCA TGT TTA ATA GAG GTA-Fc

Mismatched bases are underlined.