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Label-free DNA Y junction for bisphenol A monitoring using exonuclease III-based signal protection strategy

Junhua Chen, Shungui Zhou *

Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, China

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ABSTRACT

A label-free DNA Y junction sensing platform for the amplified detection of bisphenol A (BPA) has been constructed by the ingenious combination of toehold-mediated strand displacement and exonuclease III (Exo III)-based signal protection strategy. Three hairpin probes were utilized as the building blocks to fabricate the DNA Y junction with cascaded signal amplification *via* a series of toehold-mediated strand displacement reactions. Exo III was employed as a protecting agent for the first time to keep the Y-shaped molecular architecture intact, thereby greatly enhancing the fluorescence intensity of DNA intercalator SYBR Green I. The resulting biosensor exhibits ultrasensitivity towards BPA at low concentration (5 fM) without any labeling, modification, immobilization, or washing procedure. Our proposed sensing system also displays remarkable specificity to BPA against other possible interference molecules. Moreover, this DNA junction biosensor is robust and can be applied to the reliable monitoring of spiked BPA in environmental water samples with good recovery and accuracy. With the successful demonstration for BPA detection, the label-free DNA Y junction can be readily expanded to monitor other analytes in a simple, cost-effective, and ultrasensitive way by substituting the target-specific aptamer sequence.

1. Introduction

Bisphenol A (BPA) is an important monomer in the synthesis of polycarbonate (PC) and epoxy resins which are widely used for inner coating of feeding bottles, beverage containers, water supply pipes, and drug delivery carriers (Ragavan et al., 2013a). Tremendous amount of BPA can migrate into environment and food *via* wastewater discharge during the manufacturing process or by leaching from the packaging materials at high temperature (Geens et al., 2012). As an environmental endocrine disrupting chemical (EDC) with potential estrogenic activity, BPA can cause adverse health effects even at low concentrations (10^{-10} to 10^{-8} M) (Sheng and Zhu, 2011; Vandenberg et al., 2009). In addition, various types of cancers, cardiovascular disease, and diabetes are closely related to the intake of BPA (Tharp et al., 2012). Thus, routine detection of trace amounts of BPA with high sensitivity and selectivity is of critical importance for assuring food safety and human health.

Conventional techniques used in the quantification of BPA are mainly focused on instrument-based methods, such as high performance liquid chromatography (HPLC), liquid chromatography coupled with mass spectrometry (LC–MS), and gas chromatography coupled with mass spectrometry (GC–MS) (Ballesteros-Gomez et al., 2009). Although they offer high sensitivity and accuracy, those analytical techniques require expensive and sophisticated instrumentation, skilled operators, and time-consuming sample pre-treatment steps, which restrict their wide applications in routine measurements. To overcome these drawbacks, several groups have designed some elegant sensing strategies for colorimetric (Mei et al., 2013a; Ragavan et al., 2013b), electrochemical (Lee et al., 2011, 2014), and optical (Kuang et al., 2014; Marks et al., 2014; Zhu et al., 2015) detection of BPA by employing aptamers as the molecular recognition elements. Despite significant contributions have been made to BPA monitoring, most of these biosensors require specific labeling and immobilization procedures or extra separation and washing steps, resulting in not only a high cost of operation but also potentially complex processes. Thus, it is urgently needed to develop a label-free sensing system for BPA detection with high sensitivity, cost-effectiveness, and simplified operation.

Toehold-mediated DNA strand displacement reaction is a controllable nonenzymatic process in which one strand of DNA in a double-stranded complex is displaced by another DNA strand with the help of a short overhanging single-stranded domain (called a toehold) (Deng et al., 2014; Yin et al., 2008). Owing to its predictable thermodynamics and kinetics, this concept has been

* Corresponding author. Fax: +86 20 8702 5872.
E-mail address: sgzhou@soil.gd.cn (S. Zhou).

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successfully exploited to construct DNA nanostructures (Hariadi et al., 2015; Huang et al., 2014; Sadowski et al., 2014), molecular logic circuits (Chen et al., 2015b; Fan et al., 2015; He et al., 2014; Li et al., 2013; You et al., 2015), catalytic amplifiers (Chen et al., 2013c; Qing et al., 2014), and even neural networks (Qian and Winfree, 2011; Qian et al., 2011). Remarkably, the toehold principle also exhibits some intriguing characteristics, such as continuous signal turnover capability, inherent modularity, and easy to scale up (Zhang and Seelig, 2011; Wang et al., 2014a). These unique attributes can provide an interesting magnified sensing approach for the detection of targets with much higher sensitivity (Chen et al., 2013a,b, 2014, 2015a; Jiang et al., 2015; Song et al., 2014). However, these reported biosensing techniques usually require fluorescent labeling or base modification to provide signal output (Huang et al., 2013; Jiang et al., 2014; Lei et al., 2014; Paliwoda et al., 2014). This in turn imposes strict limitation on the application of these techniques for bioassays. Hence, using toehold-mediated strand displacement to fabricate an ultrasensitive sensing platform without any label or modification remains a compelling goal.

Exonuclease III (Exo III) is a sequence-independent enzyme that does not require a specific recognition site and can catalyze the stepwise removal of mononucleotides from 3-hydroxyl terminus of duplex DNA in the case of substrate with a blunt or recessed 3′ terminus. Its activity on single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) with a protruding 3′ end is limited. With this property, Exo III-assisted target recycle strategies were widely reported to construct amplified detectors with enhanced signals (Freeman et al., 2011; Liu et al., 2012, 2014; Tao et al., 2015; Wu et al., 2014; Wang et al., 2014b; Zhang et al., 2013, 2014). However, there is no trial using Exo III as a protecting agent in biosensor design. Herein, for the first time, we developed a label-free DNA Y junction sensing platform for BPA detection with high sensitivity and selectivity by coupling toehold-mediated strand displacement with Exo III-based signal protection strategy. With the merits of cost-effectiveness, simplicity, versatility, and ultrasensitivity, the DNA Y junction sensing system will be a promising candidate for BPA detection in environmental monitoring, food control, and clinical diagnostics.

2. Experimental

2.1. Chemicals and materials

Bisphenol A (BPA), methanol, and tris-(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich (St. Louis, MO). SYBR Green I was purchased from Life Technologies (Grand Island, NY). Exonuclease III (Exo III) (1×10^5 U/mL) was purchased from New England Biolabs (Ipswich, MA). BPA was dissolved with methanol as the stock solution and diluted using the buffer solution for analysis. Other reagents and chemicals were of analytical grade and used without further purification. All solutions were prepared with ultrapure water (18.2 MΩ/cm) from a Millipore Milli-Q water purification system (Billerica, MA).

All oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were listed in Table S1 (Supporting Information).

2.2. Construction of the DNA Y junction

The DNA solution was heated at 90 °C for 10 min and gradually cooled to room temperature at a constant rate over the course of 3 h to dissociate any intermolecular interaction. 100 nM DNA1 was initially incubated with 400 nM DNA2 in the reaction buffer (20 mM Tris–HCl, 100 mM NaCl, 25 mM KCl, 10 mM $MgCl_2$, pH=8.0) at room temperature for 30 min to form the DNA1–DNA2 duplex. BPA was then added into the above DNA1–DNA2 mixture and incubated at room temperature for 40 min to displace DNA2 and expose the toehold segment in DNA1. Subsequently, three hairpin DNA probes (hairpins A, B, and C, each was 2 μM) were added, and the mixture was incubated for 90 min at room temperature to generate the DNA Y junction structure (the A·B·C product). Finally, 35 units Exo III was added to the resulting solution, and the mixture was incubated for 30 min at room temperature.

2.3. Fluorescence detection

450 μL of the final sample solution was mixed with 50 μL of 10 × SYBR Green I solution. After incubation at room temperature for 5 min, the fluorescence spectra of the sensing system were recorded by a fluorescence spectrometer (Perkin-Elmer LS-55). The scanning wavelength ranged from 505 to 580 nm (λ_{ex}=495 nm, λ_{em}= 525 nm).

2.4. Procedure of the agarose gel electrophoresis

The 2% agarose gels contained 1 μL of ethidium bromide per 30 mL of gel volume were prepared using TBE buffer (1 ×). A volume of 8 μL of different DNA samples mixed with loading buffer (1 ×) was loaded into the lanes. Gel electrophoresis was performed at a constant potential of 100 V for 40 min and visualized under UV light by the gel image system.

2.5. Investigating the specificity of the sensor

To determine the specificity and selectivity of the developed method, several EDCs and a few other chemicals such as ethoxylated bisphenol A (BPE), bisphenol B (BPB), bisphenol C (BPC), bisphenol F (BPF), 4,4′-biphenol (4 BP), diethylstilbestrol (DES), 17β-estradiol (E2), estriol, and atrazine were tested at a concentration of 500 nM according to the procedures described above.

2.6. Analysis of actual water samples

The reliability of the sensor in practical applications was measured by determining the recovery rate in real water samples. Tap water samples were obtained from our laboratory. Lake water samples were collected from Jinkeng Reservoir (Guangzhou, China). River water samples were taken from the Pearl River (Guangzhou, China). Prior to analysis, all of the samples were filtered through a 0.22 μm membrane to remove the insoluble impurities. Aliquots of the water samples were spiked with different concentrations of BPA and diluted 5 times with the reaction buffer. Five duplicate measurements were performed for all samples. All analytical procedures were identical to those mentioned above.

3. Results and discussion

3.1. Design strategy for BPA detection

The design strategy for amplified detection of BPA on the basis of DNA Y junction and Exo III is schematically demonstrated in Scheme 1. DNA is represented as directional lines, with the squares denoting the 5′ termini and the arrows denoting the 3′ termini. The anti-BPA aptamer (Jo et al., 2011) was extended at the 5′-end to form a signal transducer strand (DNA1) where domain 1* is inhibited by hybridization with a complementary strand (DNA2). DNA2 should contain an appropriate number of bases so that it anneals to the aptamer with sufficient strength to offer low