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Dual enzyme-free amplification strategy for ultra-sensitive fluorescent detection of bisphenol A in water

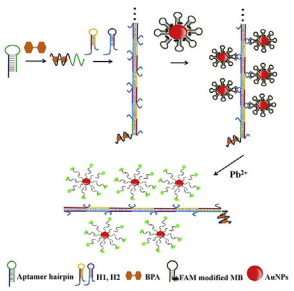
Wen Yun [a, *], Hong Wu [a], Lin Chen [c], Lizhu Yang [b, **]

[a] Chongqing Key Laboratory of Catalysis and New Environmental Materials, College of Environment and Resources, Chongqing Technology and Business University, Chongqing, 400067, China
[b] School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, Zhejiang, 325035, China
[c] State Key Laboratory of Environmental Friendly Energy Materials & School of Material Science and Engineering Southwest University of Science and Technology, Mianyang, 621010, China

HIGHLIGHTS

- An dual enzyme-free strategy for bisphenol A (BPA) detection was presented.
- This method was simple without complex enzymatic procedure and high cost.
- This method exhibited high sensitivity for BPA detection.

GRAPHICAL ABSTRACT



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ABSTRACT

An ultra-sensitive strategy for bisphenol A (BPA) detection based on dual enzyme-free strategies: hybridization chain reaction (HCR) and enzyme-strand recycling reaction has been developed. The BPA aptamer can form hairpins structure by the partly self-complementary sequence. In the presence of BPA, the released BPA aptamer sequence can trigger the HCR between two hairpins to from a long nicked double-helix DNA. The tails of hairpins on the duplex DNA were closely enough to hybridize with molecular beacon (MB) on the gold nanoparticles (AuNPs) to circularly cleave the loop of MB, leading to a "turn-on" fluorescent signal. This method exhibited high sensitivity for BPA detection in a linear rang from 0.2 to 1000 pM with 0.05 pM of limit of detection. Moreover, it was successfully used for BPA detection in real water samples. Importantly, this method was simple without complex enzymatic procedure and high cost, showing a promising future for on-site detection of BPA in practical application.

1. Introduction

Bisphenol A (BPA) was used to harden polycarbonate plastics and synthesize epoxy resin from 1950s, which is widely used for the production of feeding bottles, packaging materials, tableware and microwave ovenware [1–3]. Small amounts of BPA may migrate from the food packaging materials into food and beverages

* Corresponding author.
** Corresponding author.
E-mail addresses: 44863542@qq.com (W. Yun), yanglz3000@aliyun.com (L. Yang).

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due to thermal treatment in processing [4,5]. Because of the similar structure to endocrine hormones, which can bind to estrogen receptors, BPA is taken as an environmental endocrine disrupting chemical (EDC) which raises concerns about food safety [6,7]. Some reports reviewed that it is widely present in various food and other items, causing pollution of the biotic and abiotic environments [4,8]. The presence of BPA in the environment is not produced by naturally but the result of anthropogenic activities. It is released during the manufacturing of polycarbonate/epoxy resin and following their disposal in landfills after use. Even at very low concentration (10^{-10} to 10^{-8} M), BPA can affect human health, including metabolic disease, thyroid function, neurological effects, cancer and sexual function problems [9]. BPA have been proved that can cause effect in animal model at doses in the range of human exposes. This was lower than predicted does from some in vitro and vivo assays. Therefore, it is significantly important to develop a method for detection of BPA with high sensitivity and selectivity.

At present, many analytical techniques have been used for BPA detection, including high performance liquid chromatography (HPLC) [10,11], gas chromatography coupled with mass spectrometry (GC-MS) [12,13] and liquid chromatography coupled with mass spectrometry (LC-MS) [14,15]. These methods with high sensitivity and selectivity are the most common methods for BPA routine analysis. However, they required expensive instrument, tedious pretreatment and skilled operator which limited their potential applications area. To address these problems, various signaling transduction strategies were used for BPA detection by employing aptamers as the recognition elements including colorimetry [16,17], fluorescence [18,19], electrochemistry [20,21] and surface-enhanced Raman scattering (SERS) [22,23] et al. Although significant progresses have been made for BPA detection, it is still highly desirable to develop a strategy to detect BPA with ultra-high sensitivity for food safety and public health concerns.

In order to markedly improve the sensitivity, different strategies were used for signal amplification, including enzyme-based [24,25] or enzyme-free methods [26–28]. However, enzyme-based methods such as polymerase and exonuclease III suffered from the high cost, strict storage conditions and complexity of the experimental system, which restricted their application. Recently, enzyme-free methods attracted a lot of attention due to its significant advantages such as simple experimental design, low cost, isothermal condition and high amplification efficiency [29]. Hybridization chain reaction (HCR) firstly introduced by Dirk and Pierce is one main type of enzyme-free amplification strategy [30]. The core technology of HCR was that an initiator can triggered the self-assembly between two alternating hairpin DNA structures to form a long nicked double-helix DNA polymers. Moreover, our group developed a new enzyme-free strategy based on the enzyme-strand recycling of DNAzyme for Pb^{2+} detection [27]. This method was simple and highly sensitive without complex enzymatic procedure and extra hairpins design, showing high amplification efficiency and simple experimental design.

Here, we developed an ultra-sensitive strategy for fluorescent detection of BPA based on two kinds of enzyme-free strategies: HCR and enzyme-strand recycling. BPA aptamer was partly self-complementary to form hairpins structure. In the presence of BPA, the BPA aptamer hairpin was opened. The released BPA aptamer sequence can initiate the HCR between two alternating hairpin to form a long nicked double-helix DNA. The tails of hairpins have the similar sequence with enzyme-strand. In this condition, the tails of hairpins were closely to hybridize with the molecular beacon (MB) on the gold nanoparticles (AuNPs) to form the Pb^{2+}-dependent DNAzyme structure. It can cause the recycling catalytic cleavage of the MB on AuNPs in the presence of Pb^{2+} and lead to significant amplification of fluorescent signal for BPA detection. The limit of detection was low to 0.05 pM. This strategy has some merits such as simple, low cost and isothermal condition. This method showed ultra-high sensitivity and excellent selectivity for BPA detection. It was successfully applied to environmental water samples, showing a promising future for practical application.

2. Experimental

2.1. Chemicals and materials

BPA was brought from Zhiyuan Chemical Reagents Factory (Tianjin, China). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), 6-mercaptohexanol (MCH) and sodium citrate were purchased from Sigma-Aldrich (USA). All the DNA sequences were synthesized from Sangon Biotechnology Co., Ltd. (Shanghai, China) and purified with HPLC. The details of the sequences were shown in Table S1. Reaction buffer solution (Tris-HCl and PBS) were obtained from Beijing Leagene Biotech. Co., Ltd. (Beijing, China). All solutions were prepared with ultrapure water with resistivity of 18.2 MΩ cm.

2.2. Instruments

LS 55 fluorometer (PerkineElmer, USA) was used to measure the fluorescent intensity. The excitation and emission wavelengths of FAM were 492 nm and 517 nm respectively.

2.3. Preparation of MB-AuNPs probe

AuNPs were synthesized according to previous reported method with 13 nm average diameters [31]. Thiolated MB sequences were heated up to 95 °C for 5 min, then cooled down to room temperature slowly for MB structure formation. Then MB was added to 10 nM AuNPs to reach final concentration of 1 μM. Then, the mixture was incubated for 12 h at room temperature. Unconjugated MB was removed via centrifugation at 12,000 rpm for 15 min at 4 °C. The precipitate was washed with 50 mM Tris-HCl solution (pH 7.2). Then it was dispersed in the reaction buffer (50 mM Tris-HCl, 0.1 M NaCl, pH 7.2). Concentration of MB-AuNPs were determined via BEER-Lambert law with extinction coefficient of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ [32].

2.4. Quantitation of surface coverage of MB-AuNPs

The amounts of FAM-MB loaded on AuNPs were quantitated according to reported protocol by Demers et al. [33]. In brief, 20 mM MCH was added to FAM-MB-AuNPs solution, incubating overnight with shaking at room temperature. The released FAM-MB probes were separated via centrifugation and the fluorescent intensity was measured and the molar concentration was calculated by a standard linear calibration curve with known concentrations of FAM-MB probe.

2.5. BPA detection procedure

BPA aptamer, hairpin1 (H1) and hairpin 2 (H2) were heated up to 95 °C for 5 min, and cooled down to room temperature in 2 h for hairpin structure formation. Then BPA was added in 300 nM BPA aptamer solution and incubated for 20 min. Following that, the mixture mentioned above was added into 100 nM H1, 100 nM H2 in 100 mM PBS (pH 7.2) and 100 mM NaCl at 37 °C for 2 h HCR reaction. After that, MB-AuNPs was added for enzyme-strand recycling cleavage reaction for 30 min in 100 nM Pb^{2+}, 50 mM Tris-HCl (pH 7.2) and 0.1 M NaCl at 37 °C. Finally, fluorescent signal was measured via a quartz cuvette with 200 μL sample solution.

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