Sensors and Actuators B xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Terminal deoxynucleotidyl transferase-induced DNAzyme nanowire sensor for colorimetric detection of lipopolysaccharides

Miao Miao[a,1], Jingjing Tian[b,1], Yunbo Luo[a,b], Zaihui Du[b], Zhihong Liang[a,b,*], Wentao Xu[a,b,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, 100083, China
[b] Key Laboratory of Safety Assessment of Genetically Modified Organism (Food Safety), Ministry of Agriculture, Beijing, 100083, China

ARTICLE INFO

Article history:
Received 11 July 2017
Received in revised form
12 September 2017
Accepted 2 October 2017
Available online xxx

Keywords:
Terminal deoxynucleotidyl transferase (TdT)
DNAzyme nanowire sensor
Hybridization chain reaction (HCR)
Lipopolysaccharides (LPS)
G-quadruplexes

ABSTRACT

A new colorimetric DNAzyme nanowire sensor has been developed for the sensitive and selective quantification of lipopolysaccharides (LPS). The sensor was a label-free and isotheral reaction system based on a LPS-binding aptamer and autonomously assembled DNAzyme nanowire. In the sensing system, an aptamer-initiator (AI) was designed to recognize the LPS and initiated the hybridization chain reaction (HCR) of two DNA hairpins (H_1 and H_2). All the H_2 containing sticky ends formed terminal deoxynucleotidyl transferase (TdT) –induced G-quadruplexes. In the presence of LPS, on end of AI, and LPS-aptamer, formed the LPS/aptamer complex with the remaining LPS, the other end of AI, the initiator then started the alternate-opening of H_1 and H_2 through HCR. Furthermore, TdT-induced hemin/G-quadruplex DNAzymes along DNA nanowires were self-assembled after HCR process. As a result, the solution in light yellow was observed via the addition of H_2O_2/TMB. After optimization, the time to detect the signal was approximately 40 min, and the reaction temperature was in a broad range from 4 °C to 37 °C. The detection platform showed excellent sensitivity and selectivity for LPS from *E. coli* O111:B4 in a series of samples. In less than 2 h, a colorimetric response was achieved and the concentrations of LPS detected could be as low as 100 pg/mL. The semi-quantitative observation limit was 20 ng/mL with the naked eye. A drinking water sample was tested, which further demonstrated the feasibility of the proposed method applied for biological samples.

1. Introduction

Lipopolysaccharides (LPS), also termed endotoxins, are found on the cell envelope of all gram-negative bacteria [1], causing multiple pathogenic symptoms, such as septic shock, diarrhea, and vascular blood clotting [2]. The limulus amebocyte lysate (LAL) assay is described for LPS detection in the European and Chinese pharmacopoeia [3]. However, the LAL assay usually lasts for hours, and the appearance of β-(1,3)-D-glucan may lead to false-positive results. At present, only a few detection methods can be employed to the rapid detection of LPS including electrochemiluminescence (ECL) [4], silver stain [5], fluorescence methods [6], visible organic dyes [7], and aptasenors [2]. Among the above mentioned methods, visible dye-based staining (e.g., Procion red) is economical but has low sensitivity and is time consuming. Fluorescent stains, like EtBr, facilitate LPS detection, but the mechanism of the EtBr stain has not been understood, and the demands of expensive scanning equipment and well-trained operators have turned into burden in small-scale infirmaries. Silver staining techniques have been applied widely for LPS detection, but the use of formaldehyde as a silver reductant in this technique carries some risks [5]. ECL has high sensitivity and a rapid response to LPS detection, but the immobilization procedure of a solid-state ECL sensor is complicated. To overcome these defects, an improvement for more convenient, sensitive and selective quantification techniques for LPS detection is necessary. Therefore, the utilization of a biosensor combined with other techniques for LPS detection has attracted much attention.

Abbreviations: TdT, terminal deoxynucleotidyl transferase; LPS, lipopolysaccharides; AI, aptamer initiator; HCR, hybridization chain reaction; TMB, tetramethyl benzidine.

* Corresponding authors at: Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, 100083, China.
E-mail addresses: hongzil@sina.com (Z. Liang), xuwentao@cau.edu.cn, xuwentao1111@sina.com (W. Xu).
[1] These authors contribute equally.

https://doi.org/10.1016/j.snb.2017.10.004
0925-4005/

Table 1
DNA sequences used in this study.

Oligonucleotide	Sequence(5 '-3 ')
Aptamer-Initiator(AI)	AGCAATTGGTCCTCGCTTAGCTTCTACGGTGGGCTATCT[a]TTTTTTTTT-TCTACCTACCTAGCTCCGAATTCC[b]
$Hairpin_1$	GGCCAAACAGAATCCGAAGCTCAGACCCTGCTGAGCTTCGGATTCTGT[c]AACCG
$Hairpin_2$	CTGAGCTTCGGATTCTGTTTGGCCACAGAATCCGAAGCTCAGCAGGGT[d]

[a]Initiator of HCR. [c]Spacer sequence. [b]Aptamer of E. coli O111:B4 LPS. [cd]Hairpin sequences are referred to Ref.

With special biological functions, DNAzyme and aptamer are two typical function nucleic acid of catalysis and specific binding properties individually and usually used as biological signal amplification tools in biosensor system. DNAzymes also serves as amplifying labels for recognition reactions. Especially for the horseradish peroxidase-mimicking DNAzyme, it is composed of hemin intercalated in a G-quadruplex structure, which drew more significant attentions. Interestingly, terminal deoxynucleotidyl transferase (TdT) could produce random G-quadruplex sequences in the absence of template in a dNTP pool which consisted of dGTP and dATP in the ratio of 3:2 [8]. As for the aptamer, it is a single-stranded oligonucleotides that specifically bind to targets, such as proteins, metal ions, small molecules, and nucleic acids. Aptamer-based biosensors have gradually raised lots of concerns since they were selected by the Kim team in 2012 [9–11]. Infact, a variety of functional nucleic acids-based electrochemical, optical, and microgravimetric sensors were developed for determining DNA [12], small molecules [13], metal ions [14], proteins [15], and enzyme activity [16] in the past few years. These functional nucleic acids have been obtained through SELEX (systematic evolution of ligand by exponential enrichment) strategy [17].

Molecular amplification methods of nucleic acids such as loop-mediated isothermal amplification (LAM) [18], rolling circle amplification (RCA) [19], strand displacement amplification (SDA) [20], polymerase chain reaction (PCR) [21], and ligase chain reaction (LCR) [22] are widely used to improve the sensitivity of biosensor. However, these methods relied on template replication. Due to the possibility of cross-contamination from increased amplicons, false-positive results may occur frequently. HCR, in the corresponding kinetics-controlled events, is a cascade of self-assembly hybridization reaction between two DNA hairpins which overlap partial complementarities into a long dsDNA nanowires spontaneously in the presence of an initiator DNA [23]. Combined with other techniques such as a lateral flow nucleic acid biosensor [24], colorimetric nucleic acid assays [25,26], electrochemical nucleic acid assays [27–29], bioluminescent nucleic acid assays [30], fluorescent nucleic acid assays [31–33], and surface enhanced Raman spectroscopy (SERS) [34], HCR was further applied to molecular biology, DNA diagnostics and clinical diagnosis [35,36].

Herein, we report a novel colorimetric DNAzyme nanowire sensor for the sensitive and selective quantification of LPS by means of a LPS-binding aptamer and autonomously assembled DNAzyme nanowire. Aptamer-Initiator(AI) was designed to integrate LPS-aptamer and initiator of HCR, which not only can bind specifically to LPS but also open two hairpins. All the H_2 contained sticky ends to form terminal deoxynucleotidyl transferase (TdT) −induced G-quadruplexes with dNTP (Scheme 1). In the presence of LPS, the LPS-aptamer in one end of AI formed the LPS/aptamer complex with remaining LPS, the initiator in the other end of AI then started the alternate-opening of H_1 and H_2 through HCR. Furthermore, the TdT −induced G-quadruplex DNAzymes were self-assembled along the nicked super dsDNA concatemers nanowires after the HCR process. Hemin that acted as a ligand intercalated in a G-quadruplex structure to form a horseradish peroxidase-mimicking DNAzyme [37], catalyzed hydrogen peroxide (H_2O_2)/TMB to produce a distinct bright yellow color. The DNA sequences used in our research were shown in Table 1.

2. Materials and methods

2.1. Reagents and apparatus

All chemicals were analytical reagent grade and were employed as received. LPS from *E. coli* O111:B4 was bought from Sigma-Aldrich Co., Ltd (Shanghai, China). All synthetic DNA sequences were synthesized at Invitrogen (Beijing, China). Ultrapure water was obtained from a Millipore Milli-XQ system (Bedford, USA) and was used to dissolve purchased chemicals. The DNA marker was purchased from Tiangen Biotechnology Co., Ltd (Beijing, China). Bovine serum albumin (BSA), D-mannose, LPS from *E. coli* O55:B5 and peptidoglycan from Staphylococcus aureus, tetramethyl benzidine (TMB) were purchased from Beijing Biyuntian Co., Ltd (Beijing, China). Carboxyl-modified Nunc 96-well plates were bought from Thermo Fisher Scientific Inc. (Rochester, USA). Terminal deoxynucleotidyl transferase (TdT) was bought from New England Biolabs (Beijing, China).

Visual measurements were carried out on a portable spectrophotometer (NS810, Shenzhen 3nh Technology Co., Ltd, Shenzhen, China). The electrophoresis system consisted of a DYCP-31E vertical electrophoresis tank (Beijing Liuyi Instrument Plant, Beijing, China) and a steady voltage electrophoresis power supply (Bio-Rad, Hercules,USA). Images were recorded using a Gel Doc UV system (Bio-Rad, Hercules, USA). Isothermal titration calorimetry (ITC) was also performed (TA, Shanghai, China).

2.2. Isothermal titration calorimetry

Binding affinities of the selected aptamer to LPS were further assayed by ITC at 25 °C using a NANO ITC titration calorimetry [38]. ITC was performed using a MicroCal ITC_{200} instrument. To ensure the buffers matched, two types of samples were dissolved in the same reaction buffer (8.0 mM Na_2HPO_4, 2.5 mM NaH_2PO_4, 2.0 mM $MgCl_2$, and 0.15 mM NaCl, pH 7.4). The used samples were degassed thoroughly before measurement, and the ITC sample cell was washed with 300 μL distilled water and binding buffer successively (8.0 mM Na_2HPO_4, 2.5 mM NaH_2PO_4, 2.0 mM $MgCl_2$, and 0.15 mM NaCl, pH 7.4). The sample cell was loaded with 300 μL of aptamer solution (10 μM). The reference power of 8 μcal/s, and a syringe stirring speed of 1000 rpm were set. Titration was carried out using a 50 μL syringe filled with the LPS (100 μM). Injections started after baseline stabilization. The standard binding experiments consisted of 20 successive 2.5 μL injections every 300 s, with a 6 min interval between injections. The data were analyzed using the Origin software package and fit to a one-site binding mode.

2.3. DNAzyme nanowire sensor formation

Firstly, the two hairpins oligonucleotides were heated at 95 °C for 2 min and then cooled to 4 °C for 1 h, and 100 μL of AI (200 nM) was added to the tube. Secondly, 100 μL of 1 μM H_1 and 1 μM H_2 were added to the reaction buffer (8 mM Na_2HPO_4, 2.5 mM NaH_2PO_4, 0.15 M NaCl, 2 mM $MgCl_2$, pH 7.4) and incubated at 37 °C for 10 min. The results were verified by the agarose gel electrophoresis in 2% agarose gels at 130 V for 20 min, and then the gels were imaged. Next, 10 μL of TdT buffer, 0.3 μL dGTP (100 mM),

Please cite this article in press as: M. Miao, et al., Terminal deoxynucleotidyl transferase-induced DNAzyme nanowire sensor for colorimetric detection of lipopolysaccharides, Sens. Actuators B: Chem. (2017), https://doi.org/10.1016/j.snb.2017.10.004