Sensors and Actuators B 252 (2017) 689–696

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Enzymatic repairing amplification-based versatile signal-on fluorescence sensing platform for detecting pathogenic bacteria

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ARTICLE INFO

Article history:
Received 11 March 2017
Received in revised form 16 May 2017
Accepted 17 May 2017
Available online 18 May 2017

Keywords:
Signal-on fluorescence sensing
Homogeneous detection
Pathogenic bacteria
Target-triggered enzymatic repairing amplification

ABSTRACT

A novel fluorescence biosensing strategy for ultrasensitive and specific detection of pathogenic bacteria based on target-triggered enzymatic repairing amplification (ERA) has been developed. This strategy relies on target–aptamer binding mediated ERA reaction, which is carried out cyclically with the help of polymerase and two DNA repairing enzymes, uracil-DNA glycosylase (UDG) and endonuclease IV (Endo IV) to produce amplified fluorescence signal. In our assay, the specially designed hairpin probe (HAP) is used as DNA template responsible for producing a great quantity of reporter oligonucleotides and secondary primers, which can initiate a new cycle of polymerization-repairing amplification. Moreover, by the combination of polymerase-catalyzed incorporation of lesion bases with UDG and Endo IV-assisted ERA, multiple cycle of amplification of the recognition event is achieved, enabling ultrasensitive detection of pathogenic bacteria. Under optimal conditions, this biosensor exhibits ultrasensitivity toward target pathogenic bacteria with detection limits of 9.86 cfu mL^{-1} and a detection range of 5 orders of magnitude. Additionally, the biosensor has the ability of combating nonspecific background. Furthermore, an archer probe containing the anti-target aptamer sequence and a primer sequence is designed, which translates the binding of target to aptamer into the presence of primer sequence, enabling the detection of various targets, such as protein, DNA, small molecular, and any substance possessing its aptamer. Hence, the proposed target-triggered ERA-based signal-on fluorescence sensing strategy indeed create a versatile and useful platform for detection of pathogenic bacteria, related food safety analysis and clinical diagnosis.

1. Introduction

Foodborne pathogenic bacteria have always been a major threat to human health. In 2011, the U.S. Centers for Disease Control and Prevention reported the damage caused by foodborne diseases each year in the United States: they caused 48 million people to fall sick, 128 000 to be hospitalized, and 3000 to die [1,2]. What is more, the situation in developing countries is even more serious. Besides, the infectious dose of most pathogenic bacteria is as low as 10 colony-forming units (cfu) [3]. Thus, sensitive, robust, low cost, and ready-to-use analysis of pathogenic bacteria represents a critical approach to prevent and control foodborne diseases. Currently, the gold standard for bacterial detection remains the culture method. Though great progress in sensitivity and specificity have been made over the years, including the incorporation of chromogenic agars, the culture approach still suffers from time-consuming for routine analysis in the food industry. Culture methods require several days for pre-enrichment, enrichment, selective plating, identification, and confirmation, at which point a contaminated product could have already reached the consumer [4,5]. Thus, the standard culture methods are neither quick nor simple, making their use at the processing plant level cumbersome and limited. In recent years, various biosensing technologies, including electrochemical method [6–8], electrogenerated chemiluminescence assay [9] and colorimetric analysis [10,11], have been developed to improve the sensitivity and specificity, reduce cost and shorten analysis time in foodborne pathogenic bacteria analysis. Electrochemical-based assay method possesses the advantages of high sensitivity, but its accuracy and reproductivity are far from ideal. Electrogenerated chemiluminescence biosensor has the advantages of high sensitiv-

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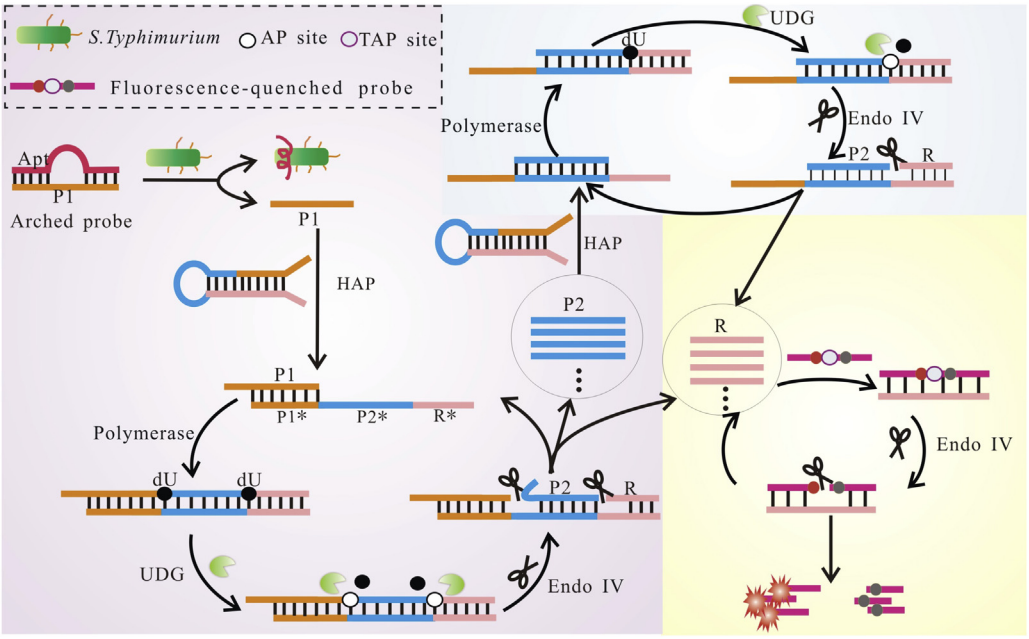
http://dx.doi.org/10.1016/j.snb.2017.05.092
0925-4005/



Scheme 1. Illustration of the target-triggered ERA for ultrasensitive fluorescence assay of *S. Typhimurium*.

Table 1
DNA oligonucleotides sequence used in this work.

Oligonucleotide name	Sequence (5' – 3 ') description
Apt	AGTAATGCCCGGTAGTTATTCAAAGATGAGTAGGAAAAGA
P1	TCTTTTCCAAAACGGGCATTACT
HAP	GCCTCCGCTTGAGCCCTGTGTCCCGTCATGCGAGTAATGCCCAAA
Fluorescence-quenched probe	GCCT(FAM)XCGC(Dabcyl)TTG

ity and ease of control, but it is not a versatile platform for analyst. Colorimetric method offers the ability for visual detection, and high portability, however, it has not satisfactory sensitivity. Thereby, it is still a challenge to develop simple, rapid, low cost and high sensitive pathogenic bacteria assay methods.

There are two factors crucial for affecting the performance of the biosensor, namely specificity and sensitivity. In order to improve the sensitivity in the detection of pathogenic bacteria, abundant nucleic acid amplification technologies is increasingly developed, such as reverse transcription quantitative PCR [12–14], rolling circle amplification [15–17], strand-displacement amplification [18–20], loop mediated isothermal amplification [21–23], hybridization chain reaction [24–26]. Most of these amplification reactions involve a key mechanism of "cleavage-replication" cyclical reaction, in which a phosphodiester bond is cleaved cyclically by nicking endonuclease such that 3′ end at the nick site can act as a primer to initiate a new cycle of extension reaction with the aid of polymerase. However, it is reported that the combination of nicking endonuclease and DNA polymerase may cause nonspecific amplification even in the absence of DNA template [27,28], possibly leading to false positive signal in the detection. Thus, it is of great significance to develop alternative mechanisms that is capable of efficient amplification via polymerase with low background.

Herein, we report the development of a novel fluorescence biosensing strategy for ultrasensitive and highly specific detection of pathogenic bacteria based on target-triggered enzymatic repairing amplification (ERA). The ERA reaction is initiated specifically by target pathogens-aptamer binding and carried out cyclically with the aid of polymerase and two DNA repairing enzymes, uracil-DNA glycosylase (UDG) and endonuclease IV (Endo IV) to produce amplified fluorescence signal. To the best of our knowledge, this is the first time that the target–aptamer binding triggered ERA has been utilized for fluorescence sensing for pathogenic bacteria. Our strategy features with several significant aspects. First, a hairpin probe (HAP) is designed to function as DNA template responsible for producing a great quantity of reporter oligonucleotides and secondary primers, which can anneal with another HAP and initiate a new cycle of polymerization-repairing amplification. Moreover, the fluorescence-quenched probe can cyclically be cleaved via Endo IV-catalyzed repairing reaction. Thus, multiple cycle of amplification of the recognition event is achieved by the combination of polymerase-catalyzed incorporation of lesion bases with UDG and Endo IV-assisted ERA, enabling ultrasensitive quantity of pathogenic bacteria. Second, the results reveal the developed ERA-based biosensing strategy could combat nonspecific background, which is probably attributed that nonspecific extension of nucleotides might incorporate too many lesions to grow into long DNA replicates. Third, the specialized design of an archer probe containing the anti-target aptamer sequence and a universal primer sequence translates the binding of target to aptamer into the pres-