Talanta 185 (2018) 141–145

Contents lists available at ScienceDirect

Talanta

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

Aligner-mediated cleavage-triggered exponential amplification for sensitive detection of nucleic acids

Wanghua Wu[a], Hongliang Fan[b], Xiang Lian[a], Jianguang Zhou[a], Tao Zhang[a,*]

[a] Research Center for Analytical Instrumentation, Institute of Cyber-Systems and Control, State Key Laboratory of Industrial Control Technology, Zhejiang University, Hangzhou 310027, PR China

[b] Department of Environmental Medicine, Institute of Hygiene, Zhejiang Academy of Medical Sciences, Hangzhou 310013, PR China

ARTICLE INFO

Keywords:
Nucleic acids detection
Isothermal exponential amplification
DNA cleavage
Aligner-mediated cleavage

ABSTRACT

Exponential amplification reaction (EXPAR), as a simple and high sensitive method, holds great promise in nucleic acids detection. One major challenge in EXPAR is the generation of trigger DNA with a definite 3′-end, which now relies on fingerprinting technology. However, the requirement of different endonucleases for varying target sequences and two head-to-head recognition sites in double stranded DNA, as well as the confinement of trigger DNA's 3′-end to be near/within the recognition site, usually subject EXPAR to compromised universality and/or repeated matching of reaction conditions. Herein, we report a simple and universal method for high sensitive detection of nucleic acids, termed aligner-mediated cleavage-triggered exponential amplification (AMCEA). The aligner-mediated cleavage (AMC) needs only one nicking endonuclease and can make a break at any site of choice in a programmable way. Thus, the 3′-end of target DNA can be easily redefined as required, a key step for initiating the amplification reaction. This capability endows the proposed AMCEA with excellent universality and simplicity. Moreover, it is sensitive and specific, with a detection limit at amol level, a broad dynamic range of 5 ~ 6 orders of magnitude and the ability to distinguish single nucleotide mutation. Experiments performed with human serum indicate that AMCEA is compatible with the complex biological sample, and thus has the potentials for practical applications.

1. Introduction

Simple, fast and sensitive detection of nucleic acids is important in many fields, such as molecular biology, medical diagnostics and forensic analysis [1–3]. To date, various methods for high sensitive detection of nucleic acids have been developed, such as surface plasmon resonance, electrochemical sensors, molecular imprinted polymer, amplification techniques, and so on [4–9]. In particular, amplification methods have attracted extensive attentions in recent years. Though polymerase chain reaction (PCR) is the most powerful tool and widely used for the quantification of nucleic acids [10], it still has some intrinsic limitations, e.g., the requirement of specific thermal-cycling instrument, considerable power consumption and relatively long reaction time. These issues usually limit its practical utility in point-of-care test. Therefore, in the past two decades, great efforts have been made in developing a variety of isothermal amplification strategies [6], such as loop-mediated amplification (LAMP) [11,12], helicase-dependent amplification (HDA) [13,14], rolling circle amplification (RCA) [15,16], smart amplification process (SMAP) [17], recombinase polymerase amplification (RPA) [18,19] and cross priming amplification (CPA) [20]. Nevertheless, most of current isothermal methods employ sophisticated mechanism or extra proteins/enzymes, which greatly increase the difficulty in primer design.

Recently, owning to the simple mechanism and high amplification efficiency, the nicking endonuclease (NEase)-based methods, such as strand displacement amplification (SDA) [21,22], exponential amplification reaction (EXPAR) [23] and nicking endonuclease-mediated isothermal amplification (NAMP) [24], have attracted extensive attentions. However, one major limitation of NEase-based methods is the requirement of specific recognition sites in target sequence, which badly restricts their universality. Many efforts, e.g., the adoption of extra bumper primers [25], fingerprinting technique [26], junction probe strategy [27] and beacon-assisted amplification [28], are either unable to address the above issue thoroughly, or likely to worsen the nonspecific background amplification that is already serious in most isothermal methods [29]. For instance, EXPAR is a simple, low-cost and sensitive method, possessing a very high amplification efficiency (10^6 to 10^9-fold amplification within minutes). Since its working principle is based on a polymerase-catalyzed extension of trigger DNA (X) along a special template to generate the double-stranded recognition site of

* Corresponding author.
E-mail address: zhtao@zju.edu.cn (T. Zhang).

https://doi.org/10.1016/j.talanta.2018.03.067
Received 29 January 2018; Received in revised form 14 March 2018; Accepted 22 March 2018
Available online 26 March 2018
0039-9140/

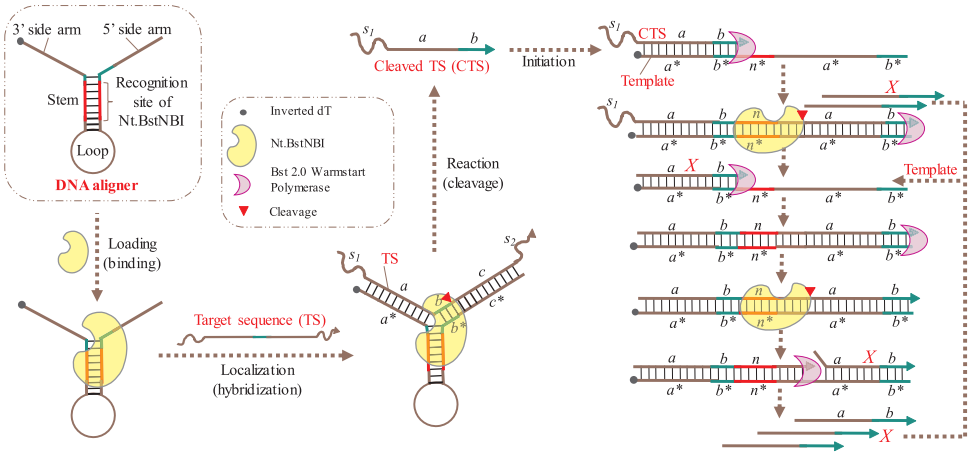

Fig. 1. Schematic illustration of AMCEA. Nt. BstNBI firstly binds the recognition site in the stem of DNA aligner (Loading), and is aligned at a specific locus through the hybridization of DA's side-arms with target DNA (Localization), and then makes a cleavage there (Reaction). Subsequently, the cleaved target serves as a trigger to initiate the EXPAR. By simply varying the sequences of aligner's side-arms, a trigger DNA can be generated from any locus of target DNA, endowing this method with excellent universality.

NEase, the main challenge to EXPAR is the generation of trigger DNA with a definite 3′-end. This process now relies on the fingerprinting technology [26], which, however, requires different endonucleases for distinct target sequences of interest, and at the same time, two adjacent recognition sites oriented head-to-head in double-stranded DNA. Except for the difficulties in choosing an appropriate endonuclease for a given sequence and the matching of reaction conditions, the trigger DNA can only be produced from double-stranded DNA, and its 3′-end must be within/near the recognition site (Fig. S1), which, to a large extent, restrains the flexibility of EXPAR from detecting the real meaningful fragment of sequences.

Our group has recently developed a simple and versatile strategy of using conventional NEases for programmable sequence-specific cleavage of DNA, termed aligner-mediated cleavage (AMC). It is based on a rationally designed hairpin-shaped DNA-aligner (DA), which consists of a recognition site of an NEase in the stem and two side arms complementary to the target sequence. Thus, an NEase can be easily loaded onto DA's stem, localized to a specific locus through the hybridization of the side arms with target DNA, and then makes a cleavage there (Fig. 1). This process needs only one NEase, does not require any special sequence in target DNA and can make a break at any intended site. In this paper, we further demonstrate an aligner-mediated cleavage-triggered exponential amplification (AMCEA). Thanks to the excellent versatility of AMC, the cleavage of target DNA, a key step to generate the trigger DNA, can be easily generated at any locus of target DNA. And the exact 3′ end can be tuned at a single-nucleotide scale by using just one NEase. This avoids the tedious processes of enzyme selection and the repeated optimization of reaction conditions. Thus, the proposed AMCEA is characterized with both excellent simplicity and universality. Moreover, this method is highly sensitive and specific, capable of detecting as less as 1.2 amol DNA and discriminating single-nucleotide mutations.

2. Materials and methods

2.1. Materials and reagents

All oligonucleotides of HPLC purity were synthesized from Sangon Biotech Co., Ltd. (Shanghai, China). Detailed sequences of these oligonucleotides are listed in Table S1. Nicking endonuclease Nt. BstNBI (10,000 U mL^{-1}), Bst 2.0 WarmStart DNA polymerase (8000 U mL^{-1}), deoxyribonucleotide triphosphates (dNTPs, 10 mM), Low Molecular Weight DNA Ladder and $MgCl_2$ solution (25 mM) were purchased from New England Biolabs (Ipswich, MA). SYBR Green I (10,000 ×) was purchased from Invitrogen Life Technologies (Carlsbad, CA). DNA-grade water (DNase- and Protease-free) and Tris-hydrochloride buffer (1 M solution, pH 8 .0) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Bovine serum albumin (BSA) and sodium chloride (NaCl) were purchased from Sigma Aldrich (St. Louis, MO). The clinical serum samples of healthy person were obtained from the Hospital of Zhejiang University. All solutions were freshly prepared with DNA-grade water.

2.2. General procedure of AMCEA

The reaction mixture of AMCEA was prepared separately on ice as part A and part B. Part A contained DA, EXPAR template, dNTPs, Nt. BstNBI and target of interest, and part B consisted of SYBR Green I and Bst 2.0 WarmStart DNA polymerase, both in the final reaction buffer (100 mM NaCl, 50 mM Tris-HCl, pH 8.0, 4 mM $MgCl_2$ and 60 μg mL^{-1} BSA). Part A was first incubated at 55 °C for 15 min on a Bio-rad CFX96 PCR system, then part B was immediately added. The final reaction mixture, composed of 50 nM DA, 50 nM EXPAR template, 6 U Nt. BstNBI, 0.4 U Bst 2.0 WarmStart DNA polymerase, 0.4 mM dNTPs, 4 mM $MgCl_2$ and 0.5 × SYBR Green I, was kept at 55 °C for 60 min and the real-time fluorescence was monitored at 1-min intervals.

2.3. Gel electrophoresis

For gel analysis, the reactions were quenched at 95 °C for 5 min at a certain time (see the black triangles in Fig. 2a and b), then 5 μL of the resultant and 20 × SYBR Green I were loaded onto 12% polyacrylamide gel, and subjected to 120 V constant voltage in 0.5 × TBE buffer (45 mM Tris, 45 mM borate, 1 mM EDTA, pH 8.3) for 50 min at room temperature. The gel was then analyzed on a Maestro Ex IN-VIVO Imaging System (CRI).

2.4. Detection of nucleic acids spiked in human serum

In order to demonstrate the utility of AMCEA in complex biological matrix, it was performed with human serum. First, the healthy human serum without any pretreatment was used to dilute target DNA by tenfold in the series. This process generated a concentration gradient from 25 fmol to 2.5 amol. Subsequently, 2.5 μL of spiked serum was subjected to AMCEA mentioned above. The results were compared with those obtained with buffered target DNA.

3. Results and discussion

3.1. Principle of AMCEA

The proposed AMCEA is illustrated in Fig. 1. The DNA aligner (DA) consists of two components: a stem-loop structure with a recognition site of Nt. BstNBI in the stem and two side arms complementary to the target sequence. First, a target sequence of interest (TS) hybridizes with DA, and is cleaved by Nt. BstNBI via AMC. Then, the cleaved target