Journal of Microbiological Methods 146 (2018) 46–50

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

Visual diagnostic of *Helicobacter pylori* based on a cascade amplification of PCR and G-quadruplex DNAzyme as a color label

Zhanmin Liu[a,*], Chenhui Yao[a], Yanming Wang[a], Wenyun Zheng[b]

[a] School of Life Sciences, Shanghai University, Shanghai 200444, PR China
[b] School of Pharmacy, Shanghai Key Laboratory of New Drug Design, East China University of Science and Technology, Shanghai 200237, PR China

ARTICLE INFO

Keywords:
Visual diagnostic
Helicobacter pylori
G-quadruplex DNAzyme

ABSTRACT

Helicobacter pylori is a spiral-shaped, Gram-negative, microaerophilic and fastidious bacterium. It is the main cause of chronic gastritis as well as gastric and duodenal ulcers. The diagnosis of *H. pylori* infection is significant for the selection of therapy and for the follow up of eradication success. A simple and robust strategy based on the cascade of PCR and DNAzyme catalyzed reaction was utilized to detect *H. pylori*. The design of the primer pair would enable PCR to synthesize aptamer of DNAzyme at the 3′ end of PCR products. G-quadruplex DNAzyme as a color label can exhibit peroxidase-like activity to amplify the specific signal and demonstrate a colorimetric signal to indicate the diagnostic result. This assay can detect genomic DNA of *H. pylori* specifically with as low as 100 pg/reaction by the naked eye. This is a powerful demonstration of G-quadruplex DNAzyme to be used for PCR-based assay with significant advantages of high sensitivity, low cost and simple manipulation over existing approaches and offers the potential opportunity for clinical application.

1. Introduction

Helicobacter pylori is a Gram-negative, microaerobic pathogenic bacteria and *H. pylori* infection is recognized as the most common cause of gastroduodenal diseases including chronic active gastritis, peptic ulcer diseases, atrophic gastritis, mucosa associated lymphoid tissue (MALT) lymphoma and noncardia gastric cancer (Dunn et al., 1997; Parsonnet et al., 1991). More than half of the world population were infected with *H. pylori*, although the majority of subjects are asymptomatic (Aziz et al., 2015). However, gastric cancer and peptic ulcer caused by *H. pylori* are responsible for more than a million deaths per year and *H. pylori* eradication can significantly reduce the incidence of metachronous gastric cancer (Kikuchi et al., 1995).

The accuracy and speed of diagnosis are as important as the availability of appropriate treatment for epidemic control. For diagnosis of *H. pylori* from gastric biopsy specimen, culturing requires particular medium in a microaerobic environment. *H. pylori* from culture medium can be detected by traditional methods based on morphological characteristics as well as urease, catalase, and oxidase tests, which are time-consuming and less sensitive.

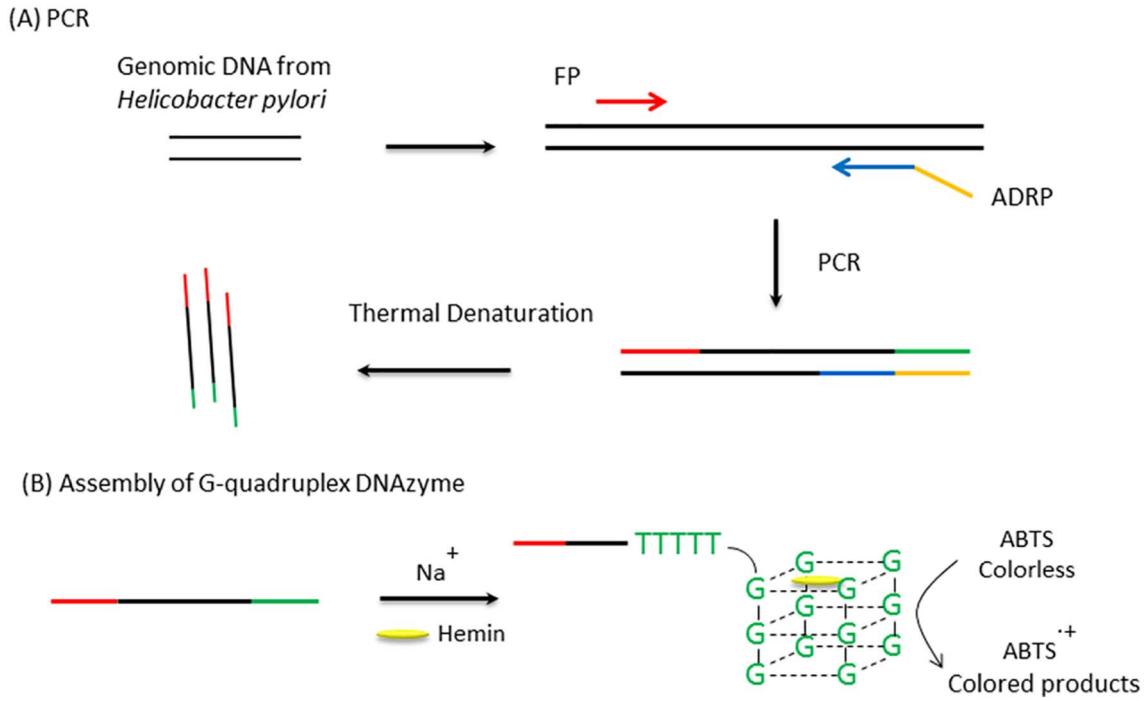
PCR is a reliable molecular technique for detection of specific pathogens, which has been widely applied to the diagnosis of *H. pylori* including detection of this pathogen in environmental samples (Enroth and Engstrand, 1995; Mapstone et al., 1993). Genes such as *Ure*A, *glm*M, *Ure*C, *16S* rRNA, *23S* rRNA, *HSP*60, and *Vac*A have been used as targets in a PCR assay (De Reuse et al., 1997; Lu et al., 1999).

The amplification techniques of DNA, such as PCR, LAMP (loop-mediated isothermal amplification) and RCA (rolling circle amplification), required endpoint analysis of the amplified products (Ge et al., 2017). Amongst the currently reported methods, the process or result of some strategies is visible to the naked eye, which achieves the concept of visual detection. Visualization of the amplified DNA from various methods, such as PCR (Polymerase Chain Reaction) (Liu et al., 2015), LAMP (Njiru, 2012) and DNA microarrays (Li et al., 2013), has been extensively applied to the identification of pathogens.

G-quadruplex DNAzyme is a kind of DNAzyme that contains a special G-quadruplex structure with an intercalated hemin, which can exhibit specific catalytic activities. G-quadruplex DNAzyme has been extensively used as a catalytic color label for various visual detection methods (Jiang et al., 2013; Li et al., 2017; Li et al., 2011; Liu et al., 2018; Wu et al., 2012). In the present work, based on the amplification efficiency of PCR, specific primers of PCR were designed to detect *H. pylori* according to *Ure*A gene (Benson et al., 2004), and the visual signal output was achieved by cascading of PCR amplification with DNAzyme catalysis. For designing the primers used in the method, the conserved *UreA* gene was determined by alignment of the sequence from multiple strains of *H. pylori* using NCBI-BLAST. The principle of our proposed method is shown in Scheme 1. The design of the primer

* Corresponding author.
E-mail address: zhmliu@shu.edu.cn (Z. Liu).

https://doi.org/10.1016/j.mimet.2018.01.014
Received 25 December 2017; Received in revised form 24 January 2018; Accepted 25 January 2018



Scheme 1. A strategy for the colorimetric detection of *Ure*A gene from *H. pylori*.

pair would enable PCR to synthesize DNA aptamer of DNAzyme at the 3′ end of PCR products. After the aptamer binding of hemin, the formed G-quadruplex DNAzyme could catalyse the H_2O_2-mediated oxidation of colorless 2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) to green-colored ABTS·+, which can further increase the signal of the existence of target DNA from *H. pylori* after PCR process.

2. Materials and methods

2.1. Materials and reagents

All DNA sequences were synthesized and purified by Sangon Biotech (Shanghai, China). ABTS, H_2O_2 and hemin were purchased from Sigma-Aldrich. *H. pylori* was obtained from Shanghai Center for Clinical Laboratory. *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Listeria monocytogenes* and *Escherichia coli* were maintained in our laboratory. *S. aureus*, *S. lugdunensis*, *L. monocytogenes* and *E. coli* were cultured aerobically on LB agar plates at 37 °C and *H. pylori* was inoculated on blood agar plates under microaerobic conditions at 37 °C.

2.2. Extraction of genomic DNA

Colonies of the *H. pylori* were washed down with physiologic saline. Genomic DNA was isolated from the bacterial suspension using the TIANamp Bacteria DNA Kit (TianGen Biotech, Beijing, China) according to the manufacturer's instructions and dissolved in ddH_2O. The extracted DNA was stored at −20 °C.

2.3. Design of primers for specific PCR product containing G-quadruplex

Based on *Ure*A gene, forward primer (FP) and reverse primer (RP) were designed to amplify the specific sequence (Table 1). The designed primers are predicted to bind to all *H. pylori UreA* genomes specifically in GenBank. The BLAST analysis revealed that they had over 96% of the identity to all *H. pylori* strains' genomes and less than 86% of the identity to other strains' genomes, which indicated the good specificity to *H. pylori*. In order to produce DNAzyme sequence for G-quadruplex DNAzyme assembly process, ADRP (anti-DNAzyme reverse primer) was designed using RP primer with anti-DNAzyme sequence attached to the 5′ end.

Table 1
Oligonucleotide primers used in this work.

Primer	Sequence (5′–3′)
FP	GGTATGCACGGTTACGAGTTT
RP	CGTGGCAAGCATGATCCAT
ADRP	CCCAACCCGCCCTACCCAAAAA CGTGGCAAGCATGATCCAT

The anti-DNAzyme sequence is underlined.

2.4. PCR procedures

The PCR reaction was performed in a reaction mixture with a final volume of 30 μL containing 1.5 mM MgCl in 20 mM Tris–HCl (pH 8.3), 20 mM KCl, 200 nM of each primer, 0.15 mM of dNTP, 1.0 U Taq DNA polymerase (Tiangen Biotechnology, Beijing, China) and 1 μL DNA template. PCR reactions were cycled using the following conditions: initial denaturation (5 min at 95 °C), 35 cycles (denaturation for 40 s at 95 °C, annealing for 30 s at 60 °C, extension for 50 s at 72 °C) and final extension for 10 min at 72 °C. The double-stranded DNA products of PCR were verified by gel electrophoresis through staining the products with ethidium bromide (EB).

2.5. Visual detection procedure

The detection system consisted of 10 mM NaCl, 4 μM hemin and previous PCR DNA products. PCR mixture was directly heated at 100 °C for 5 min. Then 4 μM hemin and NaCl were added to the mixture after the mixture was cooled down completely to room temperature. The mixture was kept at 37 °C for 20 min, and the ABTS and H_2O_2 substrates (30 μL) were added for a final concentration to be 0.9 mM (ABTS) and 1.0 mM (H_2O_2). After incubation for 5 min, the color change was observed with naked eyes and verified by UV–Visible absorption spectra.