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RESEARCH PAPER

Sensitive and Selective DNA Detection Based on Lambda Exonuclease Assisted Signal Amplification

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Abstract: A new versatile chemiluminescence biosensing platform with G-Quadruplexes/Hemin DNAzyme and lambda exonuclease (λexo) assisted signal amplification was designed for sensitive detection of DNA. The system involved target DNA, phosphate-DNA, auxiliary DNA and hairpin DNA. The selective digestion of λexo to 5′-phosphorylated strand of phosphate-DNA-auxiliary DNA-target DNA duplex sandwich structure resulted in the release of target DNA and auxiliary DNA. The released target DNA hybridized with another phosphate-DNA-auxiliary DNA duplex to trigger the target DNA recycling, while the released auxiliary DNA hybridized with hairpin DNA to activate DNAzyme segments. The activated DNAzyme segments interacted with hemin to form stable G-Quadruplexes/Hemin DNAzymes that could catalyze H_2O_2-mediated oxidation luminol to produce chemiluminescence signals. In the presence of 10 units λexo, 1.0×10^{-3} M luminol, 3.0×10^{-2} M H_2O_2 and pH 9.0 buffer solution, the relative chemiluminescence intensity of luminol was linearly related with the concentrations of target DNA in the range of 2.0×10^{-12} M to 8.0×10^{-9} M. This unique analysis strategy provided a detection limit down to 7.0×10^{-14} M. The present method was successfully applied to the determination of target DNA in serum samples, which was also capable of discriminating mismatched DNA from perfectly matched target DNA with a high selectivity.

Key Words: Lambda exonuclease; G-Quadruplexes/Hemin DNAzyme; Chemiluminescence; DNA

1 Introduction

Simple, sensitive and sequence-specific bioassay methods for the detection of trace amount DNA is of vital significance in clinical diagnosis, gene identification, environmental monitoring, food safety, as well as homeland security[1–3]. Different approaches were developed to increase the sensitivity of DNA detection by applying advanced nanomaterials, polymerase chain reaction (PCR), and rolling circle amplification (RCA) process, etc. However most of these amplification protocols were rather complex, time-consuming and expensive. Furthermore, the relatively complex handling procedures often resulted in false positive[4,5]. Recently, some alternative signal amplification strategies based on various restriction endonucleases were reported. However, these methods required specific DNA sequences, limiting their applications[6,7]. Therefore, it is still highly desirable to establish a simple, quick responsive, highly sensitive and selective detection approach for detection of trace amount DNA[8].

Lambda exonuclease (λexo) is a highly processive exodeoxyribonuclease that can selectively digest (removal of the 5′-mononucleotides) 5′-phosphorylated strand of double stranded DNA (dsDNA) in 5′→3′ direction[9,10], which plays a critical role in many vital cellular events, including DNA recombination, DNA replication and DNA repair. There are numerous examples of λexo-based amplified biosensors for detecting nucleic acid, protein and other analytes[11–14]. Most

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of these approaches, however, require chemical modification of oligonucleotide probes for the labeling of fluorescence dye, a process that is rather costly, time-consuming and sophisticated.

G-Quadruplex/hemin DNAzyme, which is composed of hemin and a single-stranded guanine-rich nucleic acid, has horseradish peroxidase (HRP)-like activity and can efficiently catalyze H_2O_2-mediated reactions, such as the oxidation reaction of 2,2′-azino-bis(3-ethylbenzothiozoline)-6-sulfonate disodium salt ($ABTS^{2-}$) by H_2O_2 to form colored product $ABTS^{\bullet-}$ or the oxidation reaction of luminol by H_2O_2 to generate chemiluminescence (CL) signal[15–17]. To date, G-quadruplex/hemin DNAzyme has shown considerable application potential as a new kind of catalytic label for the development of various biosensors in the bioanalytical field[18].

The electronic excited state of chemiluminescence (CL) technique is a product of a chemical reaction differs from fluorescence. A remarkable advantage of chemiluminescent analysis is without requiring an external light source. This is very significant in current bioanalysis because the background signal caused by an external light excitation is minimized[19–22]. Therefore, CL detection has become an attractive detection strategy. In our work, we presented a new strategy based on duplex DNA sandwich structure with three assistant DNAs and λexo assisted signal amplification for homogeneous CL detection of sequence-specific DNA. The method presented here exhibited high detection sensitivity and excellent specificity for target DNA. Furthermore, the proposed method also was successfully applied in the analysis of real biological samples.

2 Experimental

2.1 Instruments and reagents

CL spectroscopy experiments were recorded on a LS-55 luminescence spectrometer (Perkin-Elmer, USA). Oligonucleotides used in this study were synthesized and HPLC-purified by Sangon Biotechnology Co. Ltd. (Shanghai, China), and their sequences were listed in Table 1. The oligonucleotides were used and diluted in 20 mM Tris-HCl buffer solution (pH 7.4) to give stock solution of 1.0×10^{-5} M. Each oligonucleotide was heated to 95 °C for 10 min, and slowly cooled down to room temperature before use. H_2O_2 and 5-Amino-2,3-dihydro-1,4-phthalazine-dione (luminol) were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Hemin and sodium 4-(2-hy-droxyethyl) piperazine-1-ethanesulfonate (HEPES) were provided by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The lambda exonuclease (λexo) and 10 × λexo reaction buffer solutions (0.67 M Glycine-KOH (pH 9.4 @ 25 ºC), 2.0×10^{-2} M $MgCl_2$, 500 μg mL^{-1} BSA) were purchased from the New England Biolabs, Inc. (Ipswich, MA, USA). Buffer solution for CL reaction was HEPES-NH_4OH buffer (pH 7.4/8.0/8.5/9.0/9.5/10.0, 2.5 × 10^{-2} M HEPES, 0.05% (*w*/*V*) Triton X-100, 1% (*V*/*V*) DMSO, 2.0×10^{-2} M KCl, 0.2 M NaCl) solution. All other chemical reagents were of analytical reagent grade and directly used in this work. Water used throughout this work (18.2 MΩ·cm specific resistance at 25 ºC) was purified with a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA).

Table 1 Sequences of DNA used in this work

Oligonucleotide	Sequence (5′-3′)*
Phosphate-DNA	PO_4-TGT CTT GAC TCT GAC CTC ACT TCA CTT CAG GTC ATT AC
Auxiliary DNA	GTA ATG ACC TGA AGT GAA GTG
Hairpin DNA	CCC TAC CCA TTC ACT TCA GGT CAT TTG GGT AGG GCG GGT TGG G
Target DNA (TD)	AGG TCA GAG TCA AGA CA
Single-basemismatched oligonucleotide (1MT)	AGG TCA G*T*G TCA AGA CA
Three-basemismatched oligonucleotide (3MT)	AGG*A*CA G*T*G TCA *T*GA CA
Random oligonucleotide (RT)	GTAATC CGC ATC TAG TC

*, The underlined letters parts of the hairpin DNA composed of stem region. The underlined and italic letters parts represented the mismatched sites.

2.2 Method

2.2.1 Procedure for target DNA assay

The detailed procedure for target DNA detection was described as follows. First, 20 μL of target DNA at different concentration levels, 10 μL of 1.0×10^{-5} M auxiliary DNA and 10 μL of 1.0×10^{-5} M phosphate-DNA were mixed and incubated at 37 ºC for 2 h. Second, 5 μL of 2 U μL^{-1} λexo and 5 μL of 10 × λexo reaction buffer solution were added and then incubated at 37 ºC for 30 min. Subsequently, the above mixture solution was heated to 75 °C for 10 min and cooled to 37 °C and kept at this temperature for 1 h. Then, 10 μL of 1.0×10^{-5} M haipin DNA was added to the above mixture and incubated for another 30 min at 37 ºC. After incubation, the solution was diluted with 820 μL of HEPES-NH_4OH buffer solution (pH 9.0). Subsequently, 20 μL of hemin was added to the mixture at a final concentration of 2.0×10^{-7} M and incubated for 1 h at room temperature to form the G-Quadruplexes/Hemin DNAzyme structures. Finally, 50 μL of 2.0×10^{-2} M luminol and 50 μL of 0.6 M H_2O_2 were immediately added to the quartz cell containing the sample solutions obtained above, allowing the biocatalyzed oxidation of luminol to yield CL.

2.2.2 Chemiluminescence measurements

CL spectra of the sample solutions obtained above were recorded immediately with LS-55 luminescence spectrometer with a 3-mL quartz cuvette (1 cm optical path length). The light source of the spectrometer was turned off. The slits for both excitation and emission were set at 15 nm. The CL intensity was recorded at 425 nm, and the relative CL intensity ($I - I_0$, I and I_0 were the CL intensities at 425 nm in the presence and absence of target DNA, respectively) was used