Biosensors and Bioelectronics 62 (2014) 280–287

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Isothermal and rapid detection of pathogenic microorganisms using a nano-rolling circle amplification-surface plasmon resonance biosensor

Dachuan Shi, Junfu Huang, Zhengran Chuai, Dong Chen, Xiaoyan Zhu, Huan Wang, Jia Peng, Haiyan Wu, Qing Huang *, Weiling Fu *

Department of Laboratory Medicine, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China

A R T I C L E I N F O

Article history:
Received 21 April 2014
Received in revised form
25 June 2014
Accepted 25 June 2014
Available online 5 July 2014

Keywords:
Rolling circle amplification
Surface plasmon resonance
Multiplex detection
Au nanoparticles
Padlock probe
Pathogen

A B S T R A C T

Rolling circle amplification (RCA) of DNA is a sensitive and cost effective method for the rapid identification of pathogens without the need for sequencing. In this study, a surface plasmon resonance DNA biosensor based on RCA with a gold (Au) nanoparticle surface was established for isothermal identification of DNA. The probes included a specific padlock probe, a capture probe (CP), which is bound to biotin, and an Au nanoparticle-modified probe, which hybridizes with the RCA products. The CP was assembled on gold nanoparticles to increase its ability to bind and hybridize. The linear padlock probe, which was designed to circularize by ligation upon recognition of the bacterial pathogen-specific sequence in 16 S rDNA, hybridizes to fully complementary sequences within the CP. Upon recognition, each target gene DNA is distinguished by localization onto the corresponding channel on the chip surface. Then, the immobilized CPs act as primers to begin the in situ solid-phase RCA reaction, which produces long single-stranded DNA. The RCA products fixed on the chip surface cause significant surface plasmon resonance angle changes. We demonstrated that six different bacterial pathogens can be identified simultaneously and that 0.5 pM of synthetic oligonucleotides and 0.5 pg μl^{-1} of genomic DNA from clinical samples can be detected by this method with low background signals. Therefore, the multiplex diagnostic method provides a highly sensitive and specific approach for the rapid identification of positive samples.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The accurate and fast detection and identification of pathogenic bacteria are increasingly important in clinical diagnostics. Traditional techniques used to identify pathogens involve the cultivation of bacteria, which is tedious and time-consuming (Bodrossy and Sessitsch, 2004; Eriksson et al., 2009). Cultivated pathogens are identified by biochemical testing or more advanced molecular methods like PCR, which can amplify pathogen-specific nucleic acid targets (Atkins and Clark, 2004; Cho and Brennan, 2007; Lopez et al., 2003; Mahony, 2008). These approaches are effective, but they target only a single pathogen per assay.

To improve the efficiency of detection, the development of a multiplex detection method for simultaneous identification of several pathogens is needed. Surface plasmon resonance (SPR) biosensors can enable highly parallel detection of diverse organisms. SPR is an optical detection technique that uses the reflection and refraction of light. For SPR biosensors, sample solutions that contain the target molecules are injected into a running buffer that flows continuously over the sensor surface with immobilized probes (Homola, 2008). Because of the high sensitivity of the optical device, the convenient operation and the real-time monitoring, SPR biosensors offer rapid, sensitive and on-site analysis (Cosnier and Mailley, 2008). In the past decade, SPR biosensors have been used for the detection of a wide variety of bimolecular interactions, including interactions between proteins, peptides, nucleic acids, bacteria and viruses (Chu et al., 2007; Mitchell and Lowe, 2009). For a SPR biosensor chip, the mass of the molecules bound to the surface of the gold film varies proportionally to the SPR angle. Target molecules bind capture probes (CPs) and change the SPR angle (Qavi et al., 2009). SPR biosensor technology has advanced from the detection of single analytes to the performance of high-throughput screening assays (Piliarik et al., 2009). In addition, short oligonucleotides have been directly detected by SPR biosensors at amounts in the femtomole range (Springer et al., 2010), or even lower if a more complex assay method is used (Okumura et al., 2005).

Recently, the superior utility of padlock probes (PLPs) (Landegren et al., 2003; Nilsson et al., 1994) has been demonstrated for the detection and amplification of sets of target nucleic

* Corresponding authors. Tel.: +86 236 875 4429; fax: +86 236 546 0909.
E-mail addresses: Dr.Q.Huang@gmail.com (Q. Huang), fu_weiling@sina.com (W. Fu).

http://dx.doi.org/10.1016/j.bios.2014.06.066
0956-5663/© 2014 Elsevier B.V. All rights reserved.

acid sequences with high specificity and selectivity using a rolling circle amplification (RCA) mechanism (Fire and Xu, 1995; Liu et al., 1996). PLPs are long single-stranded oligonucleotide probes that contain three regions: the 5′ and 3′ ends of linear PLPs, which are designed to base pair adjacently on the target sequence; a unique sequence identifier, which binds the CP; and a universal primer binding site, which provides repeat sequences on the RCA product for Au nanoparticle-modified probe (AuNP-MP) binding. When properly hybridized adjacent to each other, the ends of PLPs can be enzymatically joined by DNA ligase. Thus, when both end segments recognize their target sequences correctly, PLPs form a circularly closed probe-target complex (Lizardi et al., 1998). Non-circularized PLPs can be removed by exonuclease treatment. The circularized PLPs can then be amplified by RCA, as observed for in vivo bacteriophage replication (Zhang et al., 2001). These circular PLPs can be amplified isothermally by DNA polymerase, which generates a long ssDNA molecule containing multiple repeats of the complementary sequence of the PLP that can reach up to several thousand-fold (Baner et al., 1998). Furthermore, the PLPs are capable of detecting single point mutations because of the necessity for precise base pairing (Nilsson et al., 1997). Consequently, the RCA technique has been applied successfully to the detection of different pathogens (Najafzadeh et al., 2011; Russell et al., 2014; Sun et al., 2011; Zhou et al., 2008).

Recently, there has been increasing interest in the use of Au nanoparticles (AuNPs) for the development of bioaffinity assays due to their unique physicochemical properties and high surface area (Manso et al., 2008). AuNPs allow stable immobilization of biomolecules due to their adhesion to metal surfaces via thiol groups (Contino et al., 2014). Notably, AuNPs can further enhance the signal amplification by RCA. AuNP-MPs that bind to RCA products strengthen the plasmon resonance of incident light excitation and improve the transduction of small refractive index changes on the chip surface, thereby increasing the sensitivity of SPR biosensors (Petryayeva and Krull, 2011). Because AuNPs serve as rapid, efficient and high-throughput platforms for detection (Su et al., 2010), they are used increasingly in SPR biosensors (Liu and Ye, 2013).

Here, we present a new strategy for multichannel SPR biosensor assay detection of multiple pathogens in parallel that has increased signal due to the use of Au nanoparticle capture probes (AuNP-CPs) and Au nanoparticle rolling circle amplification (AuNP-RCA). We designed and characterized PLPs to target specific 16S rDNA sequences of six selected bacterial species: *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Enterococcus faecalis*. The specificity and sensitivity of the multiplex assay were established for the detection of individual and mixed target pathogen DNAs. The circular PLP mixture solution, which was ligated with DNA ligase, was applied to the detection channels for hybridization with the CP, which were immobilized on AuNPs of the chip film via specific complementary sequences. Because each detection channel only had one specific CPs, the CPs hybridized with the complementary circular PLPs, which flowed into the multichannel. The other CPs were mutually used as control group, to ensure the specificity of detection. AuNP-CPs and AuNP-RCA were developed together to amplify the detection signal thrice and decrease the detection limit. The sensitivity and specificity of the SPR biosensor method were evaluated, and to verify the applicability of this approach, the method was applied to the detection of clinical samples. Our results characterize a highly sensitive and specific approach for the rapid identification of positive samples.

2. Materials and methods

2.1. Materials

Single-stranded oligonucleotides were synthesized and purified by Sangon Biotech (Shanghai, China). The sequences of these oligonucleotides are provided in Table 1. Streptavidin (SA), polyethylene glycol, dimethyl sulfoxide and mercaptoethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA), *E. coli* DNA ligase, QuickCut™ HpaI, QuickCut™ EcoRI, QuickCut™ HindIII, QuickCut™ BamHI, TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver.3.0, exonuclease I and exonuclease III were purchased from Takara (Dalian, China). Deoxyribonucleosides (dNTPs) and Phi29 DNA polymerase were purchased from Fermentas (USA). Tween 20 was purchased from Sangon Biotech (Shanghai, China). AuNPs, which were synthesized

Table 1
Sequences of oligonucleotides used in this work.

Oligonucleotide	Target species	Sequence (5′-3′)
PLPs[a]	*E. coli*	5′-pCTGTTACCGTTCGACTTGCATG*AGCACCAATAGTCTTCAGTTC***ACATCAAATGCCACG**TTAACAGTCAGGCCTAGCAAGCTTCTTC-3′
	E. faecalis	5′-pGCAAATCTCTTAAAGCTTCTCT*ACTGGTATGCTGGTTTCATCA***ACATCAAATGCCACG**TTAACAGTCAGGCCTCCGCGAGGTCAT-3′
	S. dysenteriae	5′-pCATTCTGATCCACGATTACTAGCGA*TGCTTCTTCGGTGCCCAT***ACATCAAATGCCACG**TTAACAGTCAGGCCTACGTATTCACCGTGA-3′
	S. pneumoniae	5′-pGAGTTGCGAACGGGTGAGTAA*TGTATTATCGTGTCTGCGTG***ACATCAAATGCCACG**TTAACAGTCAGGCCTTTGCTTCTCTGGAT-3′
	S. epidermidis	5′-pGGTCAGAGGATGTCAAGATT*ATACTTCACGACTCACCTCA***ACATCAAATGCCACG**TTAACAGTCAGGCCTTATCTCTAGAGG-3′
	S. aureus	5′-pAGCAAGCTTCTCGTCCGTTCGC*AGGTCATCAGTCATTCAAGTCA***ACATCAAATGCCACG**TTAACAGTCAGGCCTGCTAACATCAGAGA-3′
CPs	*E. coli*	5′biotin-CCCCCCCCCC*GAACTGAAGACTATTGGTGCT*-3′
	E. faecalis	5′biotin-CCCCCCCCCC*TGATGAAACCAGCATACCAGT*-3′
	S. dysenteriae	5′biotin-CCCCCCCCCC*ATGGGCACCGAAGAAGCA*-3′
	S. pneumoniae	5′biotin-CCCCCCCCCC*CACGCAGACACGATAATACA*-3′
	S. epidermidis	5′biotin-CCCCCCCCCC*TGAGGTGAGTCGTGAAGTAT*-3′
	S. aureus	5′biotin-CCCCCCCCCC*TGACTTGAATGACTGATGACCT*-3′
Target sequences	*E. coli*	5′- AGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGAAGCTTGCTTCTTTGCTGA-3′
	E. faecalis	5′-ATCCGAACTGAGAGAAGCTTTAAGAGATTTGCATGACCTCGCGGTCTAGCGACT-3′
	S. dysenteriae	5′-GAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGTCACGGTGAATACGTTCCCGGGCCT-3′
	S. pneumoniae	5′-CAGGTTACCTACGCGTTACTCACCCGTTCGCAACTCATCCAGAGAAGCAAGCTCCTCCTTC-3′
	S. epidermidis	5′-AACCTTACCAAATCTTGACATCCTCTGACCCCTCTAGAGATAGAGTTTTCCC-3′
	S. aureus	5′-TGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGG-3′
AuNP-MP		5′HS-$(CH_2)_6$-**ACATCAAATGCCACG**-3′

[a] Abbreviations: PLPs, padlock probes; CPs, capture probes; AuNP-MP, AU nanoparticle-modified probe. The sequences underlined in PLPs are complementary to the sequences underlined in the targets (T_1 and T_2 in Fig. 1A), while the sequences italicized in PLPs are complementary to the italicized sequences in CPs (S in Fig. 1A). The universal primer binding site (G in Fig. 1A) is indicated by bold font, and the HpaI digestion site (R in Fig. 1A) is indicated by shading. The “p” represents a phosphate at 5′ end in order to ligate the PLPs. CPs are modified with biotin at 5′ end for immobilization on the AuNPs of the gold film; the AuNP-MP is modified with a thiol group at the 5′ end for hybridization with the RCA products.

Download English Version:

https://daneshyari.com/en/article/7233144

Download Persian Version:

https://daneshyari.com/article/7233144

Daneshyari.com