Biosensors and Bioelectronics 91 (2017) 136–142

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

The analysis of proteins and small molecules based on sterically tunable nucleic acid hyperbranched rolling circle amplification

Hai Shi[a], Xiaoxia Mao[a], Xiaoxia Chen[a], Zihan Wang[a], Keming Wang[b,*], Xiaoli Zhu[a,*]

[a] Center for Molecular Recognition and Biosensing, School of Life Sciences, Shanghai University, Shanghai 200444, China
[b] Department of Oncology, The Second Affiliated Hospital of Nanjing Medical University, Nanjing 210011, China

ARTICLE INFO

Keywords:
Protein
Small molecule
Hyperbranched rolling circle amplification
Steric effect

ABSTRACT

In this work, we succeeded in establishing a new method for proteins and small molecules analysis based on the small molecule-linked DNA and nucleic acid hyperbranched rolling circle amplification (HRCA). Small molecule linked DNA by chemical modification was used as a flexible tool to study protein-small molecule interactions. The HRCA reaction which would produce signal amplification was regulated by the steric effect depending on whether the target proteins were present. In the implement of the proposed strategy, streptavidin (SA)-biotin and anti-digoxin antibody (anti-Dig)-digoxin were chosen as two model partners. Experimental results showed that the quantitative detection of SA and anti-Dig was realized both with nanomolar detection limits. The small molecules biotin and digoxin were also detected at nanomolar levels in a wide range of 1 nM~100 μM and 1 nM~10 μM, respectively. Meanwhile, the results indicated that the method had a favorable specificity in analyzing proteins or small molecules. Thus, it may be expected to quantitatively analyze some protein markers and small molecular drugs in complex biological samples.

1. Introduction

The investigation of protein-small molecule interaction is of great importance in researching the cell development (Gao et al., 2015; Ohoka et al., 2014; Wang and Yan, 2011), protein function and cell signaling networks (Miyazaki et al., 2010; Im et al., 2007). Thanks to the high affinity and specificity of the interaction, it provides a great opportunity for the analysis of proteins and small molecules. Several techniques have been developed to detect proteins based on the interactions of proteins and small molecules including colorimetry (Yang and Gao, 2014; Gong et al., 2015; Zhu et al., 2015a, 2015b), fluorescence (He et al., 2013; Ou et al., 2013; Zhou et al., 2013; Chen et al., 2016) and electrochemistry (Cao et al., 2012; Wang et al., 2013a, 2013b, 2013c; Zhao et al., 2015).

In recent years, small molecule-linked DNA is emerging as a frequently-used tool for detecting the interactions between small molecules and their protein receptors. Small molecule-linked DNA is a versatile tool, which could be as a bridge between the detection system and the DNA-triggered signal amplification system, like hybridization chain reaction (HCR) (Zhao et al., 2015; Ban et al., 2016) and rolling circle amplification (RCA) (Wang et al., 2013a, 2013b, 2013c). Thus, it can facilitate the development of detection methods for trace amounts of targets. More interestingly, the modification positions of small molecules on DNA are flexible, which triggers the surprising ideas of the researchers. For example, Jiang's group developed the proof-of-principle of a terminal protection assay of small molecule-linked DNA in 2009, which has provided a new insight for detecting small molecule-binding proteins (Wu et al., 2009). In Jiang's work, a small molecule is terminally tethered to a single-stranded DNA, different from which, Zhang's work was based on a small molecule conjugated to one of the strands in a DNA duplex (Zhang et al., 2014). Upon binding of small molecule-binding proteins, the melting temperature (T_m) of the duplex would decrease and resulted in the initiation of strand displacement reaction. The proof-of-principle could be used to detect several small molecules and their protein receptors.

In these kinds of designs, the detection of proteins is transferred to the detection of small molecules-linked DNA. The sensitivity may further be promoted by combining with nucleic acid signal amplification technologies. Motivated by such these ideas, here we developed a method for the detection of small molecules or target proteins based on sterically tunable nucleic acid hyperbranched rolling circle amplification (HRCA). HRCA is evolved from RCA and is triggered through the introduction of a second primer to hybridize with the RCA products, resulting in a turn-by-turn cascade of primer extension and strand displacement (Zhang et al., 2001). Compared to RCA, HRCA has much higher amplification efficiency (10^9-fold) and can produce numerous

* Corresponding authors.
E-mail addresses: wangkeminglab@163.com (K. Wang), xiaolizhu@shu.edu.cn (X. Zhu).

http://dx.doi.org/10.1016/j.bios.2016.12.016
Received 2 October 2016; Received in revised form 6 December 2016; Accepted 7 December 2016
Available online 08 December 2016
0956-5663/ © 2016 Elsevier B.V. All rights reserved.

long double-stranded DNA segments Lizardi et al., 1998). Therefore, HRCA is more suitable for targets analysis in low level than RCA. In this method, a small molecule was linked to the primer of HRCA at the second base from 3' terminus. In the presence of target protein, the primer was bound to the protein through the small molecule moiety, which would thereafter prevent the following HRCA because of steric hindrance. On the contrary, when free small molecules were added into the reaction system, proteins could bind with free small molecules and the primer could then trigger HRCA. The change of fluorescence intensity denoted the concentration of target proteins or small molecules. In this work, the SA-biotin system and anti-Dig-digoxin system were taken as two model pairs to test the feasibility of our strategy. The results have shown that the strategy could be employed to quantitatively and specifically analyze SA, anti-Dig and their small molecule partners.

2. Experimental section

2.1. Reagents and materials

Biotin-modified DNA strands and digoxin (Dig)-modified DNA strands were synthesized by Takara Biotechnology Co., Ltd. (Dalian, China). Other DNA oligonucleotides and agarose were obtained by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of DNA strands were listed in Table S1. SA, bovine serum albumin (BSA), ovalbumin (OVA), immunoglobulin G (IgG), thrombin, biotin, Dig, glucose, adenosine, glycine, nicotinamide adenine dinucleotide (NADH) and dopamine were purchased from Sigma-Aldrich (St. Louis, USA). Folate receptor (FR) was purchased from Sino Biological Inc. (Beijing, China), anti-Dig was obtained from Roche Diagnostics (Shanghai) Ltd. (Shanghai, China). Phi29 DNA polymerase, 10× Phi29 DNA polymerase reaction buffer and deoxynucleotides (dNTPs) solution mixture were purchased from New England Biolabs (Ipswich, MA). SYBR Green I was obtained from Solarbio Technology Co., Ltd. (Beijing, China).

2.2. Hyperbranched rolling circle amplification reaction and electrophoresis experiments

Firstly, each DNA strand was heated at 95 °C for 5 min, and gradually cooled down to room temperature before use. Then, all the following reagents were mixed including 1 μM primer 1 (P1) or small molecule-linked DNA (P1-2-B or P1-2-D), 100 nM closed circular template DNA (ccDNA), 1 μM primer 2 (P2), 1×Phi29 DNA polymerase reaction buffer, 400 μM dNTPs solution mixture and 0.2 U Phi29 DNA polymerase. And the whole mixture was incubated at 30 °C for 90 min to allow the HRCA reaction to take place. After that the temperature was raised up to 65 °C and kept for 10 min so as to inactivate the polymerase and terminate the reaction. Finally, the gel electrophoresis was performed using the HRCA products (10 μL per well) on a 1% agarose gel with the fluorescence stain SYBR Green I at 100 V for 30 min. After electrophoresis, the gel was visualized using the Gel Doc XR Imaging System.

2.3. Construction of sterically tunable hyperbranched rolling circle amplification for protein detection

The typical method was prepared by mixing different concentrations of P1-2-B (1 nM, 10 nM, 100 nM) or P1-2-D (10 nM, 100 nM) and various concentrations of target proteins in 1×Phi29 DNA polymerase reaction buffer, and incubated at 37 °C for 10 min. Subsequently, 100 nM ccDNA, 1 μM P2, 400 μM dNTPs solution mixture, 0.2 U Phi29 DNA polymerase were added into the above solution, and then the whole mixture was incubated at 30 °C for 90 min and inactivated at 65 °C for 10 min.

2.4. Construction of sterically tunable hyperbranched rolling circle amplification for small molecule detection

Different concentrations of P1-2-B or P1-2-D were incubated with equivalent concentration of small molecule-binding proteins at 37 °C for 10 min. After this, different concentrations of small molecules, biotin or Dig was added into the mixture and incubated again at 37 °C for 10 min. The following procedures for HRCA reaction are the same as that for protein detection.

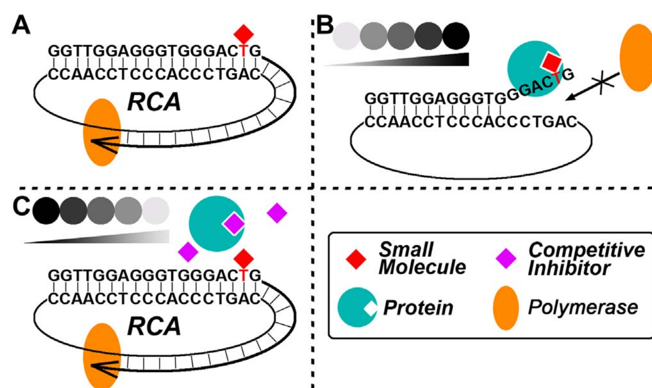
2.5. Fluorescence experiment

Before fluorescence measurements, above HRCA products and 3.2 μL 1000×SYBR Green I were mixed and incubated at room temperature for 15 min. Then the measurements were performed at ambient temperature on a Hitachi F-7000 fluorescence spectrophotometer. Both the excitation and emission slit widths were set to 5.0 nm. The emission spectra were collected with the excitation wavelength of 480 nm. The fluorescence intensity at 520 nm is used for quantitative analysis.

3. Results and discussion

3.1. The design principle of the method

As Scheme 1 illustrated, the sterically tunable HRCA was accomplished by the high affinity binding of a protein to a small molecule conjugated to the second base from 3' terminus of the primer of HRCA. In the absence of protein, the primer triggered HRCA normally and a large number of HRCA products can be obtained (Scheme 1 A). On the contrary, with the addition of target proteins, which was able to bind the small molecules conjugated to the primers, HRCA reaction was inhibited due to the large space steric hindrance of the protein (Scheme 1B). Moreover, the T_m of the primer-ccDNA hybrid duplex would decrease upon the binding of the protein (Zhang et al., 2014), which might further reduce the output of HRCA products. These are the basis of detecting target proteins. The strategy was also suitable for small molecules detection, in which situation the corresponding proteins with the same concentration of the primer are added before the small molecules were detected. As was illustrated in Scheme 1 C, in the presence of free small molecule, it would bind to the protein target, competing strongly with the small molecule modified on the primer. As a result, HRCA reaction could take place as usual. The amount of the final HRCA products were in response to the fluorescent signals and the fluorescence intensity can be used for quantitative analysis of proteins and small molecules.



Scheme 1. Schematic illustration of the principle for the quantitative detection of proteins and small molecules based on sterically tunable HRCA. Note that only rolling circle amplification (RCA) is illustrated in the scheme to simplify the graph for understanding.

Download English Version:

https://daneshyari.com/en/article/5031141

Download Persian Version:

https://daneshyari.com/article/5031141

Daneshyari.com