Talanta 186 (2018) 293–298

Contents lists available at ScienceDirect

Talanta

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

Protein binding protection in combination with DNA masking for sensitive and reliable transcription factor detection

Xiaowen Xu[a], Lei Wang[b], Desong Zhu[a], Yan Wang[c,*], Wei Jiang[a,*]

[a] Key Laboratory for Colloid and Interface Chemistry of Education Ministry, School of Chemistry and Chemical Engineering, Shandong University, 250100 Jinan, PR China
[b] School of Pharmaceutical Sciences, Shandong University, 250012 Jinan, PR China
[c] The 88th Hospital of PLA, 270100 Tai'an, PR China

ARTICLE INFO

Keywords:
Proteins binding protection
DNA masking
Transcription factor
Fluorescent detection

ABSTRACT

Sensitive and reliable detection of transcription factors (TFs) is crucial for disease diagnosis and drug discovery. Herein, a protein binding protection in combination with DNA masking-based strategy is developed for sensitive and reliable detection of NF-κB p50. First, NF-κB p50 binds specifically to a hairpin probe to form a protein-DNA complex. Then, the formed complex protects the hairpin probe from hybridization with the masking strand and subsequently triggers the cascade signal amplification of strand displacement amplification (SDA) and exponential rolling circle amplification (ERCA). Excess hairpin probes that do not bind NF-κB p50 are masked through hybridization with masking strands into stable duplexes, prohibiting the non-specific amplification and avoiding the risk of false positive signals. The signal results from the presence of NF-κB p50, ensuring the detection reliability. Through the cascade amplification specifically triggered by the target, the method can detect the purified recombinant NF-κB p50 down to 1.0×10^{-13} M. It is further employed for the NF-κB p50 inhibitor screening and analysis, which shows the potential in anti-NF-κB p50 drug discovery. Moreover, the method is applied in detection NF-κB p50 in cell nuclear extracts with a detection limit of 0.1 ng μL^{-1}. The proposed strategy will provide a promising tool for sensitive and reliable assaying NF-κB p50 activity in biomedical study and disease diagnosis.

1. Introduction

Transcription factors (TFs) are DNA-binding proteins that bind tightly to sequence-specific short double-stranded DNA segments locating in regulatory regions of genes to regulate the gene expression [1]. There are about 1400 TFs in the human cell [2]. They play crucial roles in cellular processes including DNA repair, genome replication, gene transcription and cell division [3–6]. The abnormal TFs activity is closely related with many diseases, such as inflammation, abnormal hormone responses, autoimmunity, developmental disorders and cancer [7–11]. The expression level of TFs can also indicate stages of disease development [12,13]. Some TFs have been regarded as promising biomarkers and targets for theranostics [14]. Therefore, TFs detection holds great value in disease diagnosis and drug discovery [15]. Electrophoresis mobility shift assay [16], Western blotting assay [17] and enzyme-linked immunosorbent assay [18] have been developed for the TFs detection. However, their application is partially limited by semi-quantitative analysis, the involved radioactive hazards, and the availability of antibodies against target TF.

Taking advantage of the specific binding of TFs to double-stranded DNA fragment, DNA-based sensing strategies have been proposed by flexibly integrating TF-binding sequences into synthetic DNA probes [19]. Among those, fluorescent TF sensing has drawn much attention due to its accuracy, safety and simplicity [20]. The binding of TF to the double-stranded sequence domain can transform the probe conformation and stabilize the probe in the binding-competent state, inducing the fluorescence resonance energy transfer efficiency change between the labeled fluorophores [21–23]. Besides, the TF binding can also affect other properties of a DNA probe, such as reducing its adsorption onto nanomaterials [24] and enhancing its resistance to nuclease degradation [25], which further changes the fluorescence of the probe. A major concern in these strategies is the limited sensitivity, caused by the probe conformational equilibrium that is liable to generate a relatively high background and the 1:1 mode of target recognition to signal transduction. To increase the sensitivity, the TF-bound probe is utilized as trigger to initiate the cascade amplification [26–29]. The unbound, free probes need to be digested by exonucleases with the purpose of circumventing non-specific reaction. The insufficient digestion may easily cause false positive influences [30]. Additionally, it makes difficulty to endogenous TF detection since the endogenous nucleases in

* Corresponding authors.
E-mail addresses: wy6766@sohu.com (Y. Wang), wjiang@sdu.edu.cn (W. Jiang).

https://doi.org/10.1016/j.talanta.2018.04.047
Received 9 February 2018; Received in revised form 4 April 2018; Accepted 15 April 2018
Available online 19 April 2018

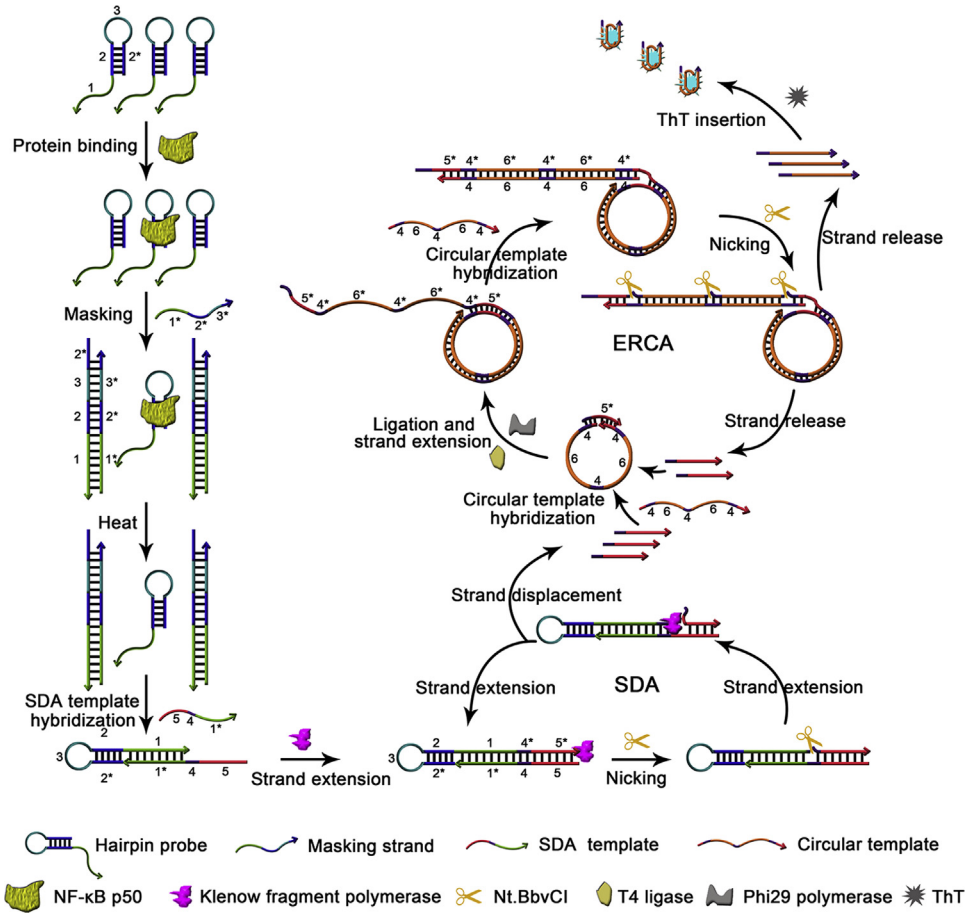

Fig. 1. Schematic illustration of protein binding protection in combination with DNA masking for transcription factor detection. The 3′-end of DNA strand is labeled with an arrow. Domains are named by numbers and complementarity is shown in the same color and denoted by asterisks. Detailed length and sequence of each domain are listed in Table S2. The name of each step of reaction is given next to the reaction arrow. SDA denotes strand displacement amplification, and ERCA denotes exponential rolling circle amplification.

real biological samples may interfere with the tool enzyme exonuclease [31]. Taking these limitations into account, it is highly desirable to develop new strategies for sensitive and reliable TFs detection without the requirement of exonucleases.

Herein, we develop an alternative fluorescent sensing strategy by combining protein binding-protection with DNA masking for sensitive and reliable detection of NF-κB p50 (Fig. 1). NF-κB p50 is a ubiquitous TF that is involved in a majority of gene regulation processes and its abnormality is closely associated with cancer [32]. First, the TF binds specifically to the hairpin probe to form a protein-DNA complex. Then, the formed complex protects the bound hairpin probe from hybridization with masking strand and triggers the cascade signal amplification of strand displacement amplification (SDA) and exponential rolling circle amplification (ERCA). Meanwhile, excess hairpin probes that do not bind the TF are masked through hybridization with masking strands into stable duplexes, prohibiting the non-specific amplification and avoiding the risk of false positive signals. Through the cascade amplification specifically triggered by the target, the method can detect the purified recombinant NF-κB p50 down to 1.0×10^{-13} M. It is further employed for the NF-κB p50 inhibitor screening and analysis, showing the potential in anti-NF-κB p50 drug discovery. Moreover, the method is applied in detection NF-κB p50 in cell nuclear extracts and obtains a detection limit of 0.1 ng μL^{-1}. The proposed strategy will provide a promising tool for sensitive and reliable assaying NF-κB p50 activity in biomedical study and disease diagnosis.

2. Experimental section

2.1. Reagents and apparatus

DNA oligonucleotides (Table S1) were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). Purified recombinant NF-κB p50 was purchased from Cayman Chemical (Ann Arbor, MI, USA). TATA-binding protein (TBP) was purchased from Abnova (Taiwan, China). HeLa cell nuclear extracts and TNF-α stimulated HeLa cell nuclear extract were purchased from Active Motif (Carlsbad, CA, USA). Klenow fragment polymerase (3′→5′ exo-, KF polymerase), T4 DNA ligase, Phi29 DNA polymerase and Nt.BbvCI were bought from New England Biolabs (Ipswich, MA). Human immunoglobulin G (IgG), human thrombin and bovine serum albumin (BSA) were bought from Sigma-Aldrich Co. (St Louis, MO, USA). Oridonin was purchased from China Langchem Inc. (Shanghai, China). Thioflavin T (ThT) was bought from Abcam (Cambridge, UK). Deoxynucleotide triphosphates (dNTPs) and 40% (w/v) acrylamide/bis-acrylamide solution (19:1) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). SYBR gold nucleic acid gel stain was purchased from Invitrogen (Carlsbad, CA, USA). All the other reagents were of analytical grade and used as received. Ultrapure water (18.25 MΩ•cm) obtained from a UP water purification system was used throughout the experiment.

The fluorescence was measured by an F-7000 spectrometer (Hitachi, Japan). The excitation wavelength was 425 nm and the spectra were recorded from 450 nm to 650 nm with a scan speed of 240 nm/min. The excitation and emission slits were both set as 5 nm, and the photomultiplier tube voltage was set as 700 V. The fluorescence intensity at 497 nm was used for quantification. The reported errors for fluorescence data represent the mean and standard deviation from three independent measurements. The gel image was photographed with GelDocTM XR^+ imaging system (Bio-RAD Laboratories Inc., USA).

2.2. Transcription factor detection procedure

The whole process includes four steps. The first step is the binding of