Biosensors and Bioelectronics 51 (2014) 255–260

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Proximity-dependent protein detection based on enzyme-assisted fluorescence signal amplification

Yuyu Tan [1], Qiuping Guo [1], Xiayu Zhao, Xiaohai Yang, Kemin Wang *, Jin Huang, Yu Zhou

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Biology, College of Chemistry and Chemical Engineering, Hunan University, Key Laboratory for Bio-Nanotechnology and Molecule Engineering of Hunan Province, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form
30 July 2013
Accepted 1 August 2013
Available online 9 August 2013

Keywords:
Proximity effect
Enzyme-assisted signal amplification
Protein detection

ABSTRACT

In this paper, we develop a sensitive fluorescence method for protein detection based on proximity extension and enzyme-assisted signal amplification. In this novel method, pairs of proximity probes are designed, and the recognition elements are integrated into the proximity probes. Then proteins are detected by transforming aptamer or antibody-protein binding signals into DNA detection based on proximity effect. In addition, nick sites are introduced into the proximity probes to amplify the detectable signal. As proof of concept, detection of human α-thrombin and human IgG are demonstrated in this study. The aptamers and antibodies are coupled in the proximity probes as recognition elements for human α-thrombin and human IgG respectively. In the presence of target protein, aptamer or antibody-protein binding signals are transformed into detectable signals by the proximity effect, and can be further amplified by enzyme-assisted strand displacement. The above mentioned strategies consequently bring the limit of detection (LOD) to as low as 1 pM for human α-thrombin and 6 pM for human IgG. Furthermore, this method might be extended to sensitive detection of other proteins by changing recognition elements of proximity probes.

1. Introduction

Many proteins are of tremendous importance in diagnosing the early stage of a disease or pathological condition (Scheuner et al., 1996; Tanzi et al., 2004). However, proteins are often present at a very low concentration, which requires highly sensitive assays. In this way, researchers usually couple the recognition elements, such as antibodies and aptamers, due to their high selectivity and affinity of binding to proteins in the field of analytical chemistry and clinical diagnostics (Liu et al., 2009; Mayer, 2009). Usually, the antibody–protein or aptamer–protein binding events are converted into amplified detectable chemical or physical signals. For example, in the past two decades, a variety of detection assays for proteins have been developed using electrochemical (Wang, 2007; Zhou et al., 2007; Zhang et al., 2007; Chikkaveeraiah et al., 2012; Gui et al., 2013), fluorescence (Xu et al., 2008; Sassolas et al., 2011; Mascini et al., 2012; Xue et al., 2012), surface plasmon resonance (Haes et al., 2005; Y. Li et al., 2006; Zhao et al., 2009; Krishnan et al., 2011; Jans and Huo, 2012; Bai et al., 2013), chemiluminescence or colorimetric sensors (Pavlov et al., 2004; D. Li et al., 2007; Wei et al., 2007; Elbaz et al., 2009; Teller et al., 2009; Zhang et al., 2011; J. Li et al., 2012; Xie et al., 2012; Qin et al., 2013). However, some of the antibodies or aptamers have relatively low association constants which limit the detection sensitivity. Thus, the developments of amplification strategies for protein sensing are crucial. Currently, the amplified detections for biorecognition events specifically for protein binding events, are still challenging in bioanalytical chemistry.

It has been a desired goal to convert the protein detection to nucleic acid detection, since high sensitivity can be achieved for the nucleic acid detection. Recent years, some DNA-based amplifications have been coupled into protein detection, such as the polymerase chain reaction (PCR) (Wang et al., 2004; Zhang et al., 2006; Xiang et al., 2007), rolling circle amplification (RCA) (Cho et al., 2005; Di Giusto et al., 2005; Yang et al., 2007), ligase chain reaction (LCR) (Barany, 1991; Gustafsdottir et al., 2007; Kim et al., 2010), hybridization chain reaction (HCR) (Dirks and Pierce, 2004; Venkataraman et al., 2007; Yin et al., 2008), strand displacement amplification (SDA) (Shlyahovsky et al., 2007; He et al., 2010; Huang et al., 2011; Qiu et al., 2011) and proximity ligation assay (PLA) (Fredriksson et al., 2002; Cheng et al., 2012). It is clear that the use of DNA-based amplification can serve to greatly enhance the sensitivity of protein detection, although some disadvantages still exist. Notably, the PLA method coupling with PCR is dependent on the simultaneous binding of the pairs of proximity probes to target, which results in a lower background signal and a high sensitivity. In addition, another DNA-based protein detection assay

* Corresponding author. Tel./fax: +86 731 88821566.
E-mail address: kmwang@hnu.edu.cn (K. Wang).
[1] These authors contributed equally to this work.

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.08.001

using nicking enzyme assisted amplification (Zheng et al., 2012), reactions can be performed under isothermal conditions without specialized instrumentation.

Herein, we develop a simpler fluorescence signal amplified method for protein detection based on enzyme-assisted strand displacement amplification, which is dependent on the simultaneous and proximal recognition of target-protein by pairs of proximity probes. As proof of concept, both human α-thrombin and human IgG are used as demonstrations. The aptamers or the antibodies are integrated into the proximity probes as recognition elements for these targets respectively. In this method, the ends of proximity probes hybridize with each other stably only when both of the proximity probes bind to the protein simultaneously. In the presence of polymerase, the hybridized probes serve as both templates and primers for strand extension. Extension of the proximity probes yields the duplex that includes the nicking sites for nicking enzyme Nb.BbvCI. In the presence of Nb.BbvCI, scission of the extension strands results in a new replication site for polymerization and the concomitant displacement of the nicked strand. In this cyclical process, an abundance of signal sequences for opening the molecular beacon can be generated by continuously repeating extension, nicking and strand displacement. This proximity-dependent enzyme assisted signal amplified method has great potential for protein detection.

2. Experimental section

2.1. Chemicals and materials

The probes and molecule beacon were synthesized by TaKaRa Bio Inc (Dalian,China). Sequences of the oligos are listed in Tables S1 and S2. Human α-thrombin, Human Immunoglobulin G (human IgG), Human Serum Albumin (HSA), and Bovine Serum Albumin (BSA) and the deoxynucleotide solution mixture (dNTPs), DTT were purchased from Dingguo Biochemical Reagents Company (Beijing, China). Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) was purchased from Thermo Fisher Scientific, Inc. Rabbit anti-human IgG and Ggoat anti-human IgG were purchased from Biosynthesis Biotechnology Co., Ltd (Beijing, China). Polymerase Klenow Fragment exo- and Nb.BbvCI were purchased from New England Biolabs, Inc. All other reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water (> 18 MΩ, Milli-Q, Millipore), and all the vessels were autoclaved.

2.2. Fluorescence measurement.

Fluorescence spectra were determined using a Hitachi F-7000 fluorescence spectrometer (Hitachi Ltd., Japan) controlled by FL Solution software. The optical path length of a quartz fluorescence cell was 1.0 cm. Excitation and emission slits were all set for a 5.0 nm band-pass. The mixtures in square quartz cuvettes were excited at 535 nm, and the emission spectra were collected from 560 to 640 nm. The fluorescence intensity at 575 nm was used to evaluate the performances of the proposed assay strategy. All samples were incubated at 37 °C. All optical measurements were performed under room temperature unless otherwise indicated.

2.3. Amplified detection of human α-thrombin

The protein determination could be briefly described as follows: upon testing various conditions, the following procedure was used to study the concentration-dependent changes in fluorescence experiments: The experiments were performed in 100 μL solution consisting of 1 μL of human α-thrombin solution of a specific concentration, 50 nM P1, 50 nM P2, 200 mM dNTPs, 200 nM MB, and buffer (10 mM Tris–HCl, pH 7.9, 10 mM $MgCl_2$, 40 mM KCl, 50 mM NaCl and 1 mM DTT) were incubated at 37 °C for 30 min to allow complete binding. Fluorescence intensity was monitored using fluorescence spectrophotometer. When the fluorescence intensity was stabilized, 10 units of Polymerase Klenow fragment exo- and 5 units of Nb.BbvCI were added to the mixture. And the proximity-triggered signal amplification was represented as the real-time fluorescence scan and wavelength scan for end-point determination.

3. Results and discussion

3.1. Design strategy for human α-thrombin detection

In the fluorescence signal amplification system, the sequences for human α-thrombin detection were designed in Table S1. Both proximity probes P1 and P2 consist of four regions (Fig. 1). Region I includes two different aptamers for human α-thrombin, which can bind to the two distinct epitopes of human α-thrombin, resulting in proximity, the green region of P1 is the Apt 29, and the yellow

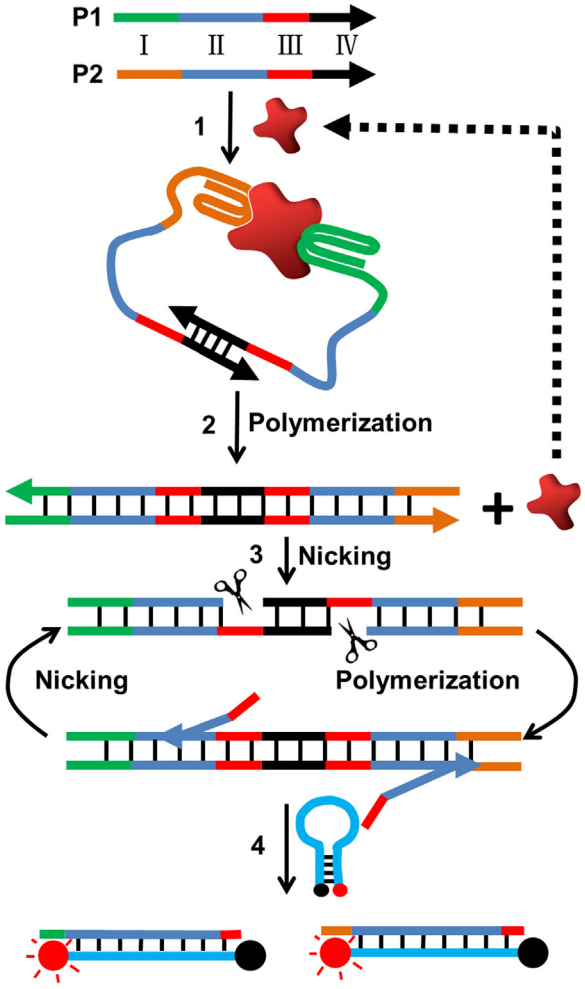

Fig. 1. (Color online) Schematic representation of the proximity-dependent and enzyme-assisted fluorescence signal amplification for human α-thrombin detection based on aptamer proximity probes. This method involves four main steps: (1) aptamer–thrombin binding; (2) proximity-induced primer extension and target-displacement amplification; (3) Nb.BbvCI nicking endonuclease assisted two nicking processes; (4) enzyme-assisted strand displacement amplifications and releasing of signal sequences. Finally, the signal sequences open the molecular beacon and restore the fluorescence signals.The green and yellow regions are the aptamers of the thrombin; the blue regions are for reading out signal; the red regions are the nick sites; the black regions are primers.