Talanta 141 (2015) 230–234

Contents lists available at ScienceDirect

Talanta

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

Label-free and homogeneous aptamer proximity binding assay for fluorescent detection of protein biomarkers in human serum

Yulian Wei [a], Wenjiao Zhou [a], Jun Liu [b], Yaqin Chai [a], Yun Xiang [a,*], Ruo Yuan [a]

[a] Key Laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China
[b] Department of Biomedical Sciences, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA

ARTICLE INFO

Article history:
Received 21 January 2015
Received in revised form
25 March 2015
Accepted 2 April 2015
Available online 9 April 2015

Keywords:
Aptamer
Label-free
Human platelet-derived growth factor
Proximity binding
Fluorescent detection

ABSTRACT

By using the aptamer proximity binding assay strategy, the development of a label-free and homogeneous approach for fluorescent detection of human platelet-derived growth factor BB (PDGF-BB) is described. Two G-quadruplex forming sequence-linked aptamers bind to the PDGF-BB proteins, which leads to the increase in local concentration of the aptamers and promotes the formation of the G-quadruplex structures. Subsequently, the fluorescent dye, N-methylmesoporphyrin IX, binds to these G-quadruplex structures and generates enhanced fluorescence emission signal for sensitive detection of PDGF-BB. The association of the aptamers to the PDGF-BB proteins is characterized by using native polyacrylamide gel electrophoresis. The experimental conditions are optimized to reach an estimated detection limit of 3.2 nM for PDGF-BB. The developed method is also selective and can be applied for monitoring PDGF-BB in human serum samples. With the advantages of label-free and homogeneous detection, the demonstrated approach can be potentially employed to detect other biomarkers in a relatively simple way.

1. Introduction

With the fast development of proteomics, it has been demonstrated that the changes in protein concentrations and altered protein expressions and distributions are associated with the occurrence and progression of different cancers [1–3]. For instance, elevated concentration of prostate specific antigen (PSA, >4.0 ng mL^{-1}) is an indication of prostate cancer [4] while increasing concentration of cancer antigen 15-3 (CA15-3, >40.0 U mL^{-1}) is related to breast cancer [5]. The identification and detection of these protein biomarkers is therefore of great importance in the early diagnosis, progression and prognosis of various cancers. The enzyme-linked immunosorbent assays (ELISAs) represent the most commonly used methods for protein biomarker detection. Although ELISAs can realize sensitive detection of different types of protein biomarkers, these methods are inherently complicated and time-consuming with the requirements of multiple washing, probe immobilization and highly trained personnel, which limit their wide application in routine clinical diagnosis [6,7]. The development of protein assays without involving complex assay protocols, to achieve homogeneous assay of proteins for example, will potentially facilitate the detection of protein biomarkers.

The proximity ligation protein assays first developed by the Landegren group have significantly simplified the monitoring of proteins [8]. In this type of assays, oligonucleotides were respectively conjugated to a pair of antibody recognition probes. When the antibodies associated with the target proteins, the conjugated oligonucleotides were brought into close proximity and hybridized with a connector oligonucleotide, which served as the ligation template to join the two termini of the conjugated oligonucleotides to form a new DNA strand with the assistance of the DNA ligase. The DNA was then subjected to real-time PCR amplification to achieve indirect and sensitive detection of the protein targets. Following this mechanism, proximity binding-induced DNA annealing [9–11] and assembly [12,13] have also been suggested as alternatives for protein detection. Indeed, the proximity binding-based assays have enabled the detection of proteins in homogenous solutions. Despite this advantage, the proximity binding assays using antibodies as the recognition probes encountered the stability and cost issues. This has intrigued the employment of aptamers as recognition probes in proximity binding assays. Aptamers with high binding specificity and selectivity to the corresponding targets [14,15] are synthetic single stranded oligonucleotides (DNA or RNA) selected from random nucleic acid libraries by the SELEX approach. Aptamers, unlike antibodies, can be

* Corresponding author. Tel.: +86 23 68252277; fax: +86 23 68254000.
E-mail address: yunatswu@swu.edu.cn (Y. Xiang).

http://dx.doi.org/10.1016/j.talanta.2015.04.005
0039-9140/

generated in vitro, which is independent on the animal immune system. Besides, aptamers can be easily manipulated and have longer shelf-life. Due to these obvious advantages, aptamers have been increasingly used as recognition probes for the detection of proteins [16], cells [17], and ions [18,19]. Considering the nucleic acid nature, aptamers are particularly suitable to be used as recognition probes in proximity binding assays [20,21]. Recently, several aptamer proximity binding assays have been reported for the detection of thrombin [22–25]. These methods show some improvements, yet, they still require the conjugation of the probes with fluorescent tags, and label-free proximity binding assays have been rarely reported.

Herein, we report on the development of an aptamer proximity binding assay for label-free and homogenous fluorescent detection of human platelet-derived growth factor BB (PDGF-BB), a critical growth factor protein dimer found in human platelets that plays important roles in regulating cell growth and division [26,27]. It can stimulate autocrine growth of different types of tumor cells and is often overexpressed in human malignant tumors [28], making PDGF-BB a potential protein marker for cancer diagnosis [29]. In our sensing strategy, the association of PDGF-BB with two binding aptamers facilitates the formation of G-quadruplex structures, which bind to the organic dye, N-methylmesoporphyrin IX (NMM) [30,31], and results in significantly enhanced fluorescent emission for sensitive detection of PDGF-BB. This approach integrates aptamer probes and G-quadruplex/NMM complexes into the proximity binding assays, thus leading to simple, convenient and sensitive detection of protein biomarkers.

2. Experimental

2.1. Materials and reagents

PDGF-BB was purchased from ExCell Biology Inc. (Shanghai, China). Tris–HCl, thrombin, mouse immunoglobin G (IgG) and lysozyme were purchased from Sigma (St. Louis, MO). The extended PDGF-BB binding aptamers (PBA1 and PBA2) and the blocking ssDNA (B-DNA) with the sequences listed in Table 1 were ordered from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). NMM was obtained from J&K Scientific Ltd. (Beijing, China). Other reagents were obtained from Kelong Chemical Company (Chengdu, China). All reagents were of analytical grade and ultrapure water (specific resistance of 18.25 MΩ cm) was used to prepare all solutions during the experimental process.

2.2. Proximity binding assay of PDGF-BB

The B-DNA/PBA1 was first prepared by mixing B-DNA (1.2 μM) with PBA1 (1.0 μM) in Tris–HCl buffer (10 mM Tris–HCl, 100 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, pH 7.4), followed by heating to 90 °C for 5 min and cooling down to 25 °C slowly in a period of 3 h.

Table 1
The sequences of the oligonucleotides used in this work.

Name	Sequence
PBA1	5′-*TAC TCA GGG CAC TGC AAG CAA TTG TGG TCC CAA TGG GCT GAG TAT* TTT TTT TTT TTT TTT TTT TTT TTTCTG CTA GGT CT**G GGT AGG G**-3′
PBA2	5′-TT**G GGC GGG** TGA CCT AGC AAT TTT TTT TTT TTT TTT TTT TTT *ACT CAG GGC ACT GCA AGC AAT TGT GGT CCC AAT GGG CTG AGT A*-3′
B-DNA	5′-CAT AGC GAG ATC CAG ACC TAG CAG-3′

*The italic, bold and underlined sequences, respectively, indicated the PDGF-BB binding aptamers, G-quadruplex forming sequence and the complementary sequences.

The mixture of B-DNA/PBA1 (50 nM) and PBA2 (50 nM) was then incubated with various concentrations of PDGF-BB in Tris–HCl buffer for 45 min at 25 °C. This was followed by the addition of NMM (2 μM) and further incubation of the mixture for 30 min. Finally, the fluorescence intensities of the mixtures were measured on a RF-5301-PC spectrophotometer (Shimadzu, Tokyo, Japan) with the excitation at 399 nm. The fluorescence emission spectra were collected from 580 nm to 650 nm with the slit widths for excitation and emission both at 10 nm.

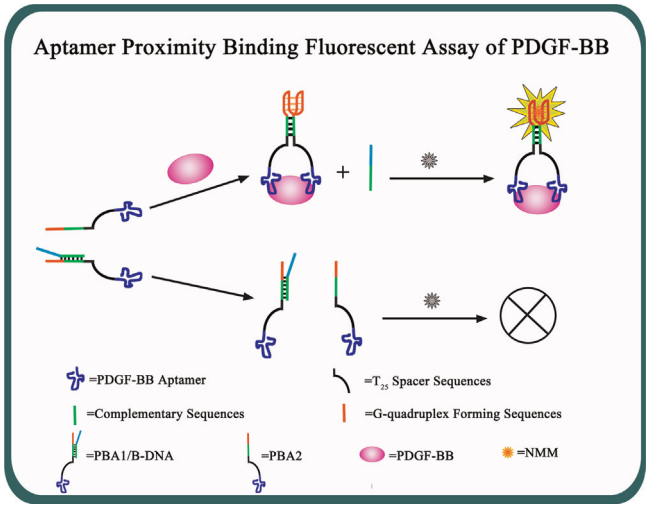
2.3. Native polyacrylamide gel electrophoresis (PAGE)

The sample solutions were subjected to electrophoresis analysis on the 16% native polyacrylamide gel. Electrophoresis was carried out in 1 × TBE (pH 8.3) at a 100 V constant voltage for 90 min. The gels were then stained with ethidium bromide for 10 min, followed by photographing with a digital camera under UV irradiation.

3. Results and discussion

3.1. Principle for fluorescent detection of PDGF-BB

Our aptamer proximity binding assay approach for fluorescent detection of PDGF-BB involves three oligonucleotide sequences: two extended PDGF-BB binding aptamers (denoted as PBA1 and PBA2) and one blocking ssDNA (B-DNA). Extra sequences are linked to the PDGF-BB binding aptamers to validate the assay protocol while not affecting the binding affinity of the aptamers to PDGF-BB. The G-quadruplex forming sequence employed to generate the signal output is split into two segments (the red regions in Scheme 1), which are separately integrated into PBA1 and PBA2. The probes for PDGF-BB detection are thus composed of four regions (Scheme 1): the G-quadruplex forming sequences (the red regions), the complementary sequences (the green regions), the T_{25} spacer sequences (the black regions) that eliminate the steric hindrance to the hybridization between the complementary sequences when binding to PDGF-BB, and the original PDGF-BB binding sequences (the blue regions). The B-DNA is hybridized to the green region and part of the G-quadruplex forming sequences in PBA1 to prevent hybridization between the complementary



Scheme 1. Illustration of the label-free and homogeneous aptamer proximity binding assay for fluorescent detection of PDGF-BB. (For interpretation of the references to color in this scheme, the reader is referred to the web version of this article.)