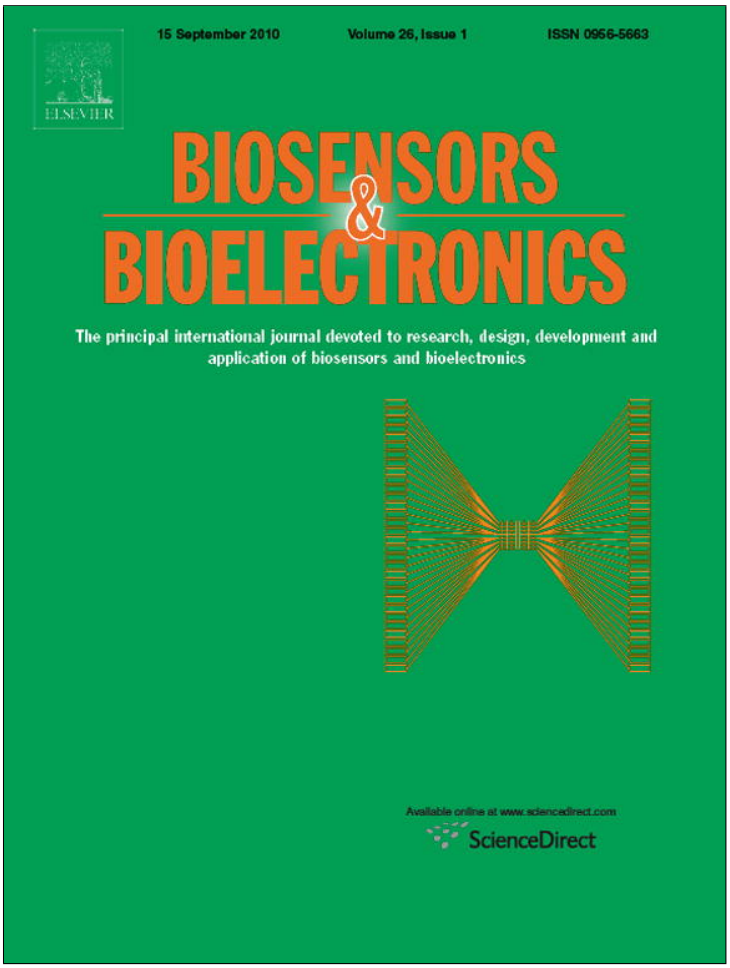

Biosensors and Bioelectronics 26 (2010) 285–288

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

A new kind of aptamer-based immunomagnetic electrochemiluminescence assay for quantitative detection of protein

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ARTICLE INFO

Article history:
Received 25 March 2010
Received in revised form 4 June 2010
Accepted 20 June 2010
Available online 25 June 2010

Keywords:
Aptamer
Immunomagnetic
electrochemiluminescence
Protein
Platelet-derived growth factor B-chain

ABSTRACT

A new kind of aptamer-based immunomagnetic electrochemiluminescence (IM-ECL) assay for quantitative detection of protein is developed. The assay consists of a double aptamer sandwich format in which a biotin-labeled aptamer is used for rapid and specific capture of target protein, and a $[Ru(bpy)_3]^{2+}$ (TBR)-labeled aptamer is used for ECL detection. As an example, platelet-derived growth factor B-chain homodimer (PDGF-BB) was detected by the method. Experimental results show that the detection limit of the assay is 80 pmol/L of PDGF-BB. A calibration curve with a linearity range from 0.1 to 1000 nmol/L is established, thus, make quantitative analysis possible. The method has been used to detect PDGF-BB in fetal calf serum with minimum background interference. Due to the wide availability of aptamer for numerous proteins, this new method holds great promise in protein detection.

1. Introduction

Proteins play very important roles in almost all functions of life. Assays for specific and sensitive detection of proteins and their molecular variants are necessary in many biotechnological applications and biomedical studies. The most commonly used method for protein detection is enzyme-linked immunosorbent assay (ELISA). However, the assay requires multiple steps and long incubation time (≥1 h per step), further more, the result is semi-quantitative. To overcome these shortcomings, an immunomagnetic electrochemiluminescence (IM-ECL) assay has been developed, in which a biotin-labeled antibody is used for rapid and specific capture of target protein by streptavidin microbeads through biotin–streptavidin linkage, and a ruthenium (II) tris-bipyridal ($[Ru(bpy)_3]^{2+}$, TBR)-labeled antibody is used to react with tripropylamine (TPA) to emit light for ECL detection. Though the IM-ECL assay can detect target protein with high sensitivity, rapidness and wide dynamic range (Blackburn et al., 1991; Yan et al., 2004; Miao, 2008), the usage of antibodies in the assay introduced some drawbacks, such as antibody is temperature-sensitive, irreversible denaturation, and has a limited shelf life. These disadvantages limit the application of current IM-ECL assay.

With the further study of the structure–function relationship of nucleic acid molecules, a new kind of artificial oligonucleotides is beginning to emerge as a class of molecules that rival antibodies in both diagnostic and therapeutic applications. These oligonucleotides, referred to as "aptamers", are generated via SELEX (systemic evolution of ligands by exponential enrichment) technique. They can fold into well-ordered, three-dimensional structures to recognize a variety of targets including proteins, peptides, small molecules, and even intact viruses and cells (Tuerk and Gold, 1990; Ellington and Szostak, 1990; Jayasena, 1999). Aptamers share similar identification principle for targets with antibodies; further more, they can provide several advantages over antibodies, such as simple synthesis, easy labeling, good stability, reversible thermal denaturation, unlimited shelf life, wide target range, and high sensitivity. Therefore, since the first application of aptamer to detect human vascular endothelial growth factor by Drolet in 1996 (Drolet et al., 1996), various aptamer-based biosensors have been established, including quartz crystal microbalance, surface plasma resonance (SPR), fluorescence, electrochemistry, ECL, and colorimetry (Luzi et al., 2003; Tombelli et al., 2005; Zhou et al., 2007; Liu et al., 2009). In these aptamer-based biosensors, ECL sensors are very promising due to the combination of advantages of both electrochemical and chemiluminescent biosensors, such as high sensitivity and easy control.

Recently, Zhang's group has reported a highly sensitive aptamer-based ECL biosensor for protein detection using electrode surface as solid support (Li et al., 2007). However, the procedures of immobilizing aptamer to the electrode surface and releasing aptamer from the electrode surface were complicated and time-consuming. Using magnetic beads instead of the electrode surface as solid support will conquer these defects since magnetic beads can provide a fast and simple selection of target protein from com-

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doi:10.1016/j.bios.2010.06.028

plex matrix, and the immobilization and release of aptamer only need to activate or deactivate the magnetic field (Centi et al., 2008).

In the present study, we try to use magnetic bead as solid support to develop a new kind of aptamer-based IM-ECL assay. To test the feasibility of the assay, we applied it to analyze platelet-derived growth factor B-chain homodimer (PDGF-BB), a growth factor protein found in human platelets (Huang et al., 2005).

2. Experimental section

2.1. Materials and reagents

Fetal calf serum, TPA and the chemicals to synthesize $[Ru(bpy)_3]^{2+}$ N-hydroxysuccinimide ester (TBR-NHS ester) were purchased from Sigma (Louis, MO, USA). Streptavidin microbeads (2.8 μm diameter) were products of Dynal Biotech (Lake Success, NY, USA). Recombinant human PDGF-AA, PDGF-AB, and PDGF-BB were purchased from R&D Systems, Inc. DNA oligonucleotides modified with biotin and amino group were synthesized by Sangong Inc. (Shanghai, China) (sequence 1: 5′-biotin-AAAAAAAAA-ATACTCAGGGCACTGCAAGCAA TTGTGGTCCCAATGGGCTGAGTAT-3′; sequence 2: 5′-amino-AAAAAAAAAATACTCA GGGCACTGCAAGC AATTGTGGTCCCAATGGGCTGAGTAT-3′). The underlined sequences represent the aptamer sequences specific for PDGF-BB, which are identified by Green et al. via the SELEX process from a library of 3×10^{14} molecules (500 pmol) of single-stranded DNA randomized at 40 contiguous positions (Green et al., 1996). The 5′-amino modified aptamer was label with TBR-NHS ester by our lab according to Terpetschnig's paper (Terpetschnig et al., 1995).

2.2. Aptamer-based IM-ECL assay

A custom-built ECL detection system was described in detail in our previous research (Zhu et al., 2004, 2008). For the samples analysis, 20 μl Tris–HCl buffer (pH 7.1, 20 mmol/L Tris–HCl, 140 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$) containing 1 μmol/L biotin-labeled aptamer (sequence 1) and 1 μmol/L TBR-labeled aptamer (sequence 2) was incubated with 10 μL of PDGF-BB in PBSM buffer (137 mmol/L NaCl, 10.1 mmol/L Na_2HPO_4, 1.8 mmol/L KH_2PO_4 (pH 7.4), 2.7 mmol/L KCl, 1 mmol/L $MgCl_2$, and 10 g/L bovine serum albumin) at room temperature for 20 min. The solution was then transferred into the electrochemical detection cell and incubated with streptavidin microbeads at 37 °C for 30 min with gentle shaking to form the biotin–streptavidin linkage. The microbeads were washed with phosphate-buffered saline (PBS, pH 7.4) twice in the magnetic field, resuspended in 100 μl ECL assay buffer (0.2 M NaH_2PO_4, 0.04% Tween 20, 0.1 M TPA, pH 8) and detected by ECL assay. The applied potential of the ECL reaction was 1.25 V according to previous optimization (Zhu et al., 2004).

2.3. Calibration of the aptamer-based IM-ECL assay for PDGF-BB

To prepare the calibration standards, three separate samples for each PDGF-BB concentration (0.01 nmol/L–10 μmol/L) were prepared and analyzed, respectively. To further evaluate the reproducibility of the assay, each sample was measured 30 times with 1-s data integration, and the averages and standard deviations were calculated using Microsoft Excel spread sheet function. The calibration curve was plotted as the ECL value (counts per second, cps) against the PDGF-BB concentrations in PBSM.

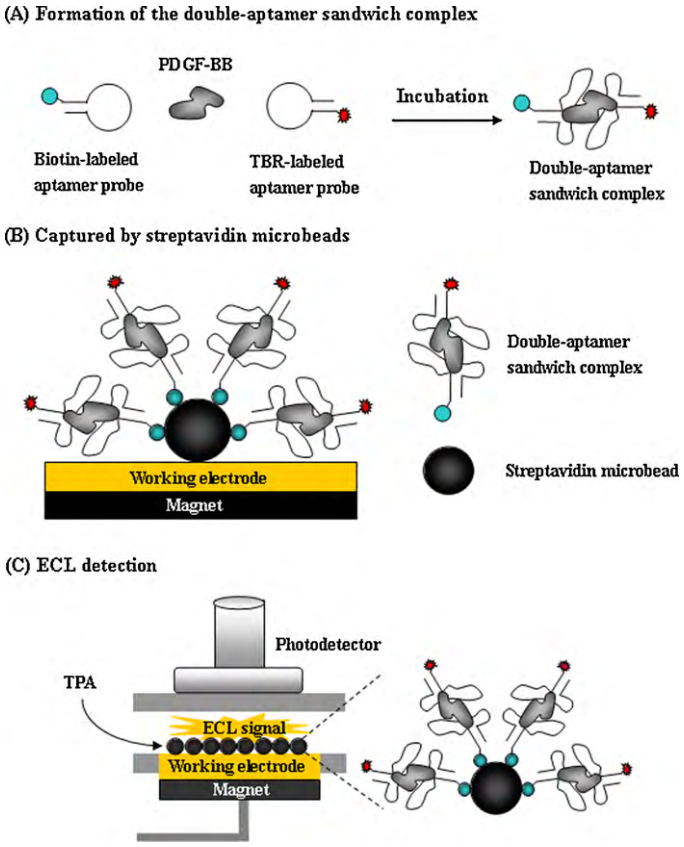

Fig. 1. Schematic illustration of the aptamer-based IM-ECL assay.

3. Results and discussion

3.1. Experimental approach

Fig. 1 shows the basic principle of the aptamer-based IM-ECL assay. The biotin-labeled aptamer and the TBR-labeled aptamer specific for different epitopes of PDGF-BB bind simultaneously with the target protein, to form a double-aptamer sandwich complex (A). The sandwich complex is then captured by the streptavidin microbeads through biotin–streptavidin linkage (B). The amount of PDGF-BB is determined by measuring the ECL signal generated from the electrochemical reaction of TBR and TPA in the reaction cell (C).

3.2. Specificity of the aptamer-based IM-ECL assay

To test the specificity of the aptamer-based IM-ECL assay, PDGF-AA homodimer, PDGF-AB heterodimer and PDGF-BB homodimer were detected by the assay, respectively. PDGF-AA and PDGF-AB are 2 other PDGF isoforms. They were each incubated with the PDGF-BB aptamer and processed in the same way as PDGF-BB. Results show that: the ECL values of PDGF-AA and PDGF-AB are close to the ECL value of blank control; while the ECL value of PDGF-BB is significantly higher than the ECL value of blank control (Fig. 2). These results demonstrate that the aptamer-based IM-ECL assay is highly specific; it has a good selectivity for discrimination of PDGF-BB from other proteins.

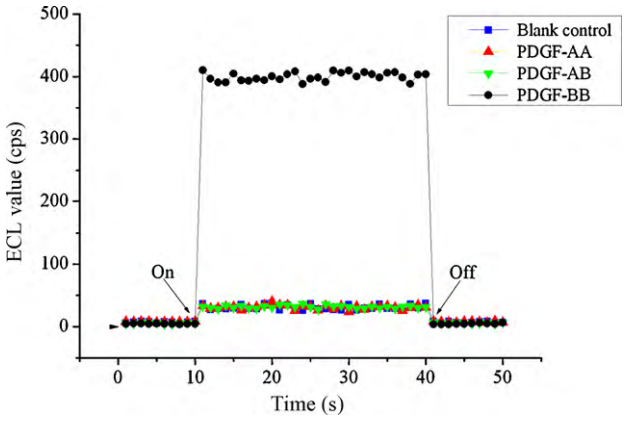

Fig. 2. Detection of PDGF-AA, PDGF-AB and PDGF-BB showing the specificity of the aptamer-based IM-ECL assay. On: potentiostat on. Off: potentiostat off.

3.3. Repeatability and reproducibility of the aptamer-based IM-ECL assay

To estimate the repeatability and reproducibility of the aptamer-based IM-ECL assay, three separate samples for each PDGF-BB concentration (0.01 nmol/L–10 μmol/L) were prepared and each sample was measured 30 times with 1-s data integration. The results are shown in Fig. 3(A), each bar represents an average value based on 30 1-s measurements from the same sample, with the error bar (standard deviation) reflecting the measurement variation of the system. The variations in ECL value, among the three separately prepared samples at each PDGF-BB concentration level, reflect the variations introduced by an operator during each assay. It can be concluded, based on the data, that the assay can detect protein with excellent repeatability and reproducibility.

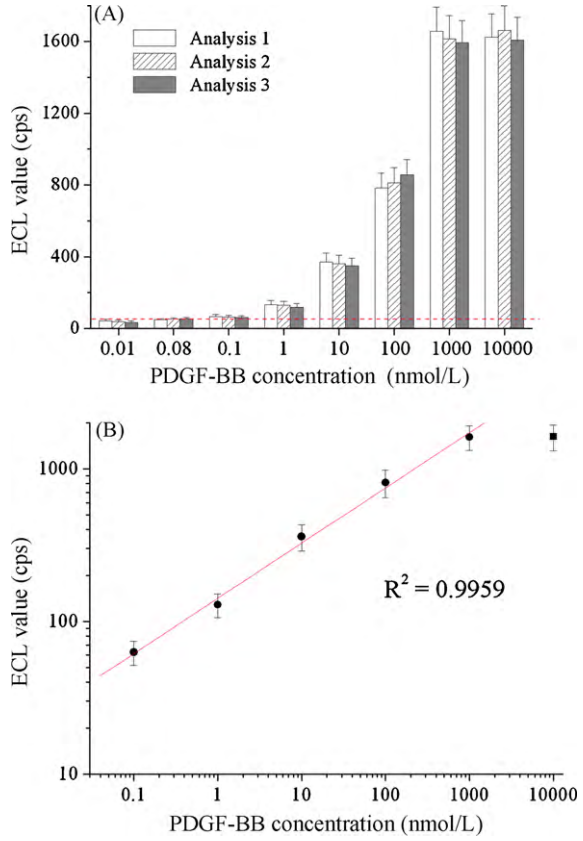

Fig. 3. (A) Repeatability and reproducibility of the aptamer-based IM-ECL assay. The cutoff value is indicated as dashed lines. Each analysis represents a single run with 30 s acquisition time of each concentration on each day. Error bars indicate standard deviations from 30 s acquisition time. (B) A standard linear regression (log–log) curve. The linear regression is come from solid circles. The solid circles represent the average ECL values from three independent assays. $R^2 = 0.9959$.

3.4. Calibration of the aptamer-based IM-ECL assay for PDGF-BB

Fig. 3(A) shows that ECL value increases with the PDGF-BB concentration. When the PDGF-BB concentration reached 0.08 nmol/L, we observed the ECL value approximately the same as the projected cutoff value of 50.12 cps, which is calculated as the average signal of blank control + three times the standard deviation of blank control (3 SD). It is reasonable to assume this value (0.08 nmol/L) as the detection limit of PDGF-BB concentration for our detection system.

The calibration curve is plotted in a double log scale, as shown in Fig. 3(B). The linear regression is come from the solid circles, which represent the average ECL values from three independent assays. Linear regression of the data is performed for PDGF-BB concentrations between 0.1 and 1000 nmol/L ($R^2 = 0.9959$). When the PDGF-BB concentration is greater than 1000 nmol/L, the ECL value does not rise with the increased PDGF-BB concentration. This prozone effect may be due to the overloaded amount of the aptamers. For our particular application, the dynamic range of our assay is enough for quantitative analysis of PDGF-BB. If the ECL value is higher or equal to the highest point of the linear portion, the unknown samples can be diluted for a proportional measurement.

3.5. Detection of PDGF-BB in fetal calf serum

The aptamer-based IM-ECL assay was further applied to analyze PDGF-BB in spiked fetal calf serum samples. The extraction recoveries of PDGF-BB from serum samples spiked with 0.5, 2, 10, 50, and 200 nmol/L were measured based on the calibration curve using pure PDGF-BB in PBSM buffer at comparable levels. Five replicates for each concentration were detected by the assay. Table 1 shows the statistical data for the quantization of PDGF-BB. The data indicated the recoveries ranged from 90 to 100% and the coefficients of variation were less than 10%.

The ECL values of PDGF-BB measured in serum were lower than the signal measured in PBSM buffer (recoveries <100%). This weak matrix effect could be owing to the presence of some matrix components in solutions which might bind or modify part of the protein, so as to decrease its availability for binding to the aptamers. Similar results were reported previously (Centi et al., 2007). However, the aptamer-based IM-ECL assay developed in our study is content to detect PDGF-BB in complex samples, particularly in terms of specificity and sensitivity.

Aptamer-based assays can be set up in a variety of formats (sandwich or competitive assays, direct or indirect assays). Centi et al. (2008) have demonstrated that the sandwich format can give a better specificity and sensitivity than other formats. Therefore, a sandwich format was used in our assay. The biotin-labeled aptamer is used for rapid and specific capture of the target protein and the TBR-labeled aptamer is used for ECL detection. The high affinity of

Table 1
Recoveries of PDGF-BB from the spiked fetal calf serum samples determined by the aptamer-based IM-ECL assay ($n = 5$).

Amount added (nmol/L)	Amount measured (nmol/L)	Recovery (%)
0.5	0.46 ± 0.02	92.0
2	1.92 ± 0.10	96.0
10	9.65 ± 0.25	96.5
50	45.8 ± 1.6	91.6
200	193.6 ± 8.4	96.8

the biotin–streptavidin interaction ($K_d = 10^{-15}$) allows for only the target protein captured by both the biotin-labeled aptamer and the TBR-labeled aptamer can be detected. Therefore, the assay should be highly specific in theory. The good specificity of the assay has also been demonstrated by experiment, as shown in Fig. 2.

The innovative aspect of the aptamer-based IM-ECL assay is the coupling of aptamers with magnetic beads for the target protein immobilization before the ECL detection. Magnetic beads are a powerful tool in a variety of bioassays (Rye and Nustad, 2001). They can rapidly and simply select low-abundance target analytes (DNA, protein, bacteria) from complex samples without any pre-enrichment or purification steps. The usage of magnetic beads instead of the electrode surface as solid support makes our assay rapid, simple and specific. Aptamers have many advantages over antibodies. Their application makes our assay reagent-stable and low cost.

4. Conclusion

In conclusion, a new kind of aptamer-based IM-ECL assay is developed. The assay is specific, sensitive, wide dynamic range, rapid, simple, reagent-stable and low cos. It can potentially become a powerful tool for protein detection due to the wide availability of aptamer for numerous proteins. Quantitative information for target proteins can be obtained by the calibration curve.

Acknowledgments

This research is supported by the National Natural Science Foundation of China (30600128), the Natural Science Foundation of Guangdong Province (7005825), the Program for Changjiang Scholars and Innovative Research Team in University (IRT0829), the Key Program of NSFC-Guangdong Joint Funds of China (U0931005), the National Basic Research Program of China (2010CB732602), and the National High Technology Research and Development Program of China (863 Program) (2007AA10Z204).

References

Blackburn, G.F., Shah, H.P., Kenten, J.H., Leland, J., Kamln, R.A., Link, J., Peterman, J., Powell, M.J., Shah, A., Talley, D.B., Tyagl, S.K., Wilkins, E., Wu, T.G., Massey, R.J., 1991. Clin. Chem. 37, 1534–1539.
Centi, S., Tombelli, S., Minunni, M., Mascini, M., 2007. Anal. Chem. 79, 1466–1473.
Centi, S., Messina, G., Tombelli, S., Palchetti, I., Mascini, M., 2008. Biosens. Bioelectron. 23, 1602–1609.
Drolet, D.W., Moon-McDermott, L., Roming, T.S., 1996. Nat. Biotechnol. 14, 1021–1025.
Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822.
Green, L.S., Jellinek, D., Jenison, R., Ostman, A., Heldin, C.H., Janjic, N., 1996. Biochemistry 35, 14413–14424.
Huang, C., Huang, Y., Cao, Z., Tan, W., Chang, H., 2005. Anal. Chem. 77, 5735–5741.
Jayasena, S.D., 1999. Clin. Chem. 45, 1628–1650.
Li, Y., Qi, H., Peng, Y., Yang, J., Zhang, C., 2007. Electrochem. Commun. 9, 2571–2575.
Liu, J., Cao, Z., Lu, Y., 2009. Chem. Rev. 109, 1948–1998.
Luzi, E., Minunni, M., Tombelli, S., Mascini, M., 2003. Trends Anal. Chem. 22, 810–818.
Miao, W., 2008. Chem. Rev. 108, 2506–2553.
Rye, P.D., Nustad, K., 2001. BioTechniques 30, 290–295.
Terpetschnig, E., Szmacinski, H., Malak, H., Lakowicz, J.R., 1995. Biophys. J. 68, 342–350.
Tombelli, S., Minunni, M., Mascini, M., 2005. Biosens. Bioelectron. 20, 2424–2434.
Tuerk, C., Gold, L., 1990. Science 249, 505–510.
Yan, G., Xing, D., Tan, S., Chen, Q., 2004. J. Immunol. Methods 288, 47–54.
Zhou, L., Ou, L.J., Chu, X., Shen, G.L., Yu, R.Q., 2007. Anal. Chem. 79, 7492–7500.
Zhu, D., Tang, Y., Xing, D., Chen, W.R., 2008. Anal. Chem. 80, 3566–3571.
Zhu, D., Xing, D., Shen, X., Liu, J., Chen, Q., 2004. Biosens. Bioelectron. 20, 448–453.