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Aptamer-based portable biosensor for platelet-derived growth factor-BB (PDGF-BB) with personal glucose meter readout

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ABSTRACT

A novel portable biosensor for sensitive and selective detection of platelet-derived growth factor-BB (PDGF-BB) had been developed based on the specific recognition between aptamer and protein using a personal glucose meter (PGM) as readout. In the presence of PDGF-BB, the primary aptamer of PDGF-BB which is bound to the surface of streptavidin magnespheres paramagnetic particles (SA-PMPs) reacts quantitatively with invertase-functionalized secondary aptamer of PDGF-BB to form a stable complex, resulting in the attachment of invertase on the SA-PMPs. Subsequently, the invertase catalyzes the hydrolysis of sucrose to produce a large amount of glucose and quantitative readout by the PGM. The enhanced signal of the PGM has a relationship with the concentration of PDGF-BB in the range of 1.0×10^{-14} M ~ 3.16×10^{-12} M, and the detection limit is 2.9 fM. The proposed portable biosensor had been successfully applied to assay the PDGF-BB in saliva samples.

1. Introduction

The personal glucose meter (PGM) is a successful device for point-of-care testing, which has wide accessibility to the public worldwide due to a great deal of advantages including the portable "pocket" size, cost effectiveness, reliable quantitative results and easy operation (Carroll et al., 2007). However, the application of this successful portable sensor is still limited in its single target (glucose) and its dynamic range of 10–600 mg/dL or 0.6–33 mM glucose (Clark and Lyons, 1962; Montagnana et al., 2009). Recently, different approaches have been developed to expand the application of PGM. For example, Xiang and Lu (2011, 2012a, 2012b, 2013) developed some sensors to monitor a series of non-glucose targets by taking advantage of invertase-labeled functional DNAs and antibodies. Yan et al (2013) reported a target-responsive "sweet" hydrogel combined with a commercial glucose meter for cocaine and adenosine triphosphate detection. Su et al.(2013) established a sensitive copper(II) sensor which combined the merits of PGM and click chemistry. We also developed a convenient histidine sensor based on the strategy of click chemistry by using PGM as readout (Zhou et al., 2014).

Platelet-derived growth factor-BB (PDGF-BB) plays a vital role in regulating cell growth and division. It is a necessary protein for increased healing coupled with fibroblast activation and granulation tissue formation in the treatment of some human chronic dermal wounds (Pierce et al., 1995). Hence, the detection of PDGF-BB in clinical biochemical research works is very important. Various methods have been applied in PDGF-BB determination, such as fluorescence (Qiu et al., 2011), luminescence (Babu et al., 2013), colorimetric (Tang et al., 2012) and electrochemical (Wu et al., 2010). Also most of these methods own the character of high sensitivity, but relatively expensive equipment and trained operators are needed, which is limited in a remote area. So developing a method for PDGF-BB determination based on simple device is important.

Aptamer is a kind of functional nucleic acid macromolecules which is made up of single-stranded DNA/RNA molecules that can be bind to a variety of non-nucleic acid target molecules with high specificity and affinity (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Many selective aptamer-based PDGF-BB biosensors had been earlier reported. For example, (Tang et al., 2012) designed a simple colorimetric sandwich sensor for PDGF-BB detection with a low detection limit via dual enzyme processes to significantly amplify the signal, but the procedure was complicated. Wu et al. (2010) developed a new electrochemical aptameric sensing system for the detection of PDGF-BB with high selectivity, but the detection limit is not low enough. Moreover, these methods have only been operated effectively and

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successfully in the laboratory. They are difficult to monitor online, or use in personal families due to their large scale and expensive equipment. Thus, the development of a more sensitive, low-cost, simple and portable biosensor for PDGF-BB determination is still a challenge.

In this study, a convenient aptamer-based biosensor for PDGF-BB determination by using the PGM as readout has been developed, which combined the characters of high selectivity of aptamer and convenience and cost-effectiveness of PGM. The proposed biosensor had been applied to detect PDGF-BB in clinical human saliva samples with satisfactory results.

2. Experimental sections

2.1. Materials and reagents

Platelet-derived growth factor-BB (PDGF-BB) was purchased from Biosynthesis Ltd. (Beijing, China). 4-(N-Maleimidomethyl) cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester sodium salt (Sulfo-SMCC), 6-mercaptohexanol, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), invertase (β-fructosidase, from baker′s yeast) and Tween-20 were achieved from Sigma-Aldrich (St. Louis, MO, USA). NaH_2PO_4 and Na_2HPO_4 were bought from Shanghai Chemical Reagent Company (Shanghai, China). All other reagents were of analytical reagent grade or better and were used directly without further purification. Streptavidin magnespheres paramagnetic particles (SA-PMPs) were purchased from Promega Corporation (Madison, USA). A commercially available glucose meter (ACCU-CHEK Avia) and test strips were received from Roche Diagnostics India Pvt. Ltd. (Mannheim, Germany) and used at room temperature. The photographs were taken with a Panasonic DMC-FX35 digital camera (Panasonic, Japan). The sequence of PDGF-BB-binding primary aptamer and secondary aptamer (left to right: 5′ to 3′) used herein was synthesized and purified by Shanghai Sangon Biotech. Co. Ltd. (Shanghai, China). The sequence is shown below:

3′-biotin modified primary aptamer:

CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTTTT-Biotin

3′-thiol group modified secondary aptamer:

CAGGCTACGGCACGTAGAGCATCACCATGATCCTGTTTT–$(CH_2)_3$–HS

Milli-Q reagent water (Milli-Q, Millipore, 18.2-MΩ resistivity) was used throughout the process. Other buffer solutions used in this study are shown below:

Buffer A: 0.1 M NaCl, 0.1 M sodium phosphate buffer solution, pH 7.4.

Buffer B: 0.1 M NaCl, 0.1 M sodium phosphate buffer solution, pH 7.4, 0.05% Tween-20.

The sucrose solution (1.0 M) is prepared in the buffer A solution (pH 7.4) and stored at 4 °C.

2.2. Synthesis of capture probe

Firstly, a portion of 0.6 mL of 1 mg/mL SA-PMPs was washed thrice by buffer B solution and then dispersed in 0.6 mL of buffer B solution. Biotin modified primary aptamer was added into the solution to achieve a final concentration of 20 μM; then the mixture was placed on a shaker for 1 h at room temperature. The specific combination of streptavidin with biotin (Weber et al., 1989) allowed the combination of SA-PMPs with biotin modified primary aptamer. SA-PMPs with primary aptamer were separated from the mixture by a magnet and then further rinsed by buffer B solution thrice. Finally, they were resuspended in buffer B solution and stored at 4 °C for further use.

2.3. Preparation of detection probe

The invertase-labeled secondary aptamer was prepared according to the previous report (Hermanson, 2008) with a little modification. In brief, 30 μL of 100 μM thiol modified secondary aptamer was dissolved in Millipore water, 2 μL of 3 mM TCEP and 20 μL of 10 mM sodium phosphate buffer at pH 5.5 were added into the Millipore water, and then mixed and incubated for 1 h at room temperature. Finally, the mixture was dialyzed against buffer A solution to obtain the thiol-secondary aptamer. For invertase conjugation, 800 μL of 10 mg/mL invertase solution and 1 mg of Sulfo-SMCC were added into buffer B solution, after incubation and vibration for 5 min; the mixture was mixed on a shaker for 1 h at room temperature. Note that the mixture was then centrifuged and the insoluble excess Sulfo-SMCC was separated from the mixture. The clear solution of Sulfo-SMCC-activated invertase was mixed with the thiol modified secondary aptamer and then incubated at room temperature for 48 h. To remove the excrescent thiol modified secondary aptamer, the solution was dialyzed against buffer A solution to obtain detection probe.

2.4. Procedures for PDGF-BB detection

The measurement is performed in 30 μL buffer B solution (pH 7.4) containing detection probe (4 μM) and capture probe (4 μM). In the absence of PDGF-BB, very low PGM signals can be detected; the color of strips has no obvious change. The background PGM signal is about 0.6 mM. Different concentrations of PDGF-BB had been added into the above mixture and incubated for 1.5 h in a dark drawer at room temperature to induce the conjunction of two aptamers and target. After that, SA-PMPs with invertase were separated from the mixture by a magnet to remove the excrescent matter. Then the SA-PMPs with invertase were further washed with buffer B solution (50 μL) thrice and eventually suspended in 10 μL buffer B solution. Subsequently, 20 μL of the sucrose solution (1.0 M) was added into the solution carefully and kept at room temperature for 20 min. The enzymatic reaction generated glucose and then detected by the PGM. The data of PGM after and before the addition of PDGF-BB was collected for the quantitative analysis. All the measurements were repeated thrice and the standard deviation was calculated for the error analysis.

3. Results and discussion

3.1. The principle of the portable biosensor for PDGF-BB

The principle of the portable biosensor for PDGF-BB is shown in Fig. 1. Firstly, SA-PMPs with primary aptamer was formed as the capture probe and invertase-labeled secondary aptamer was regarded as the detection probe. In the absence of PDGF-BB, a weak interaction occurred between the two probes, resulting in a weak signal from the PGM. In the presence of PDGF-BB, the combination of the capture probe and the detection probe leads

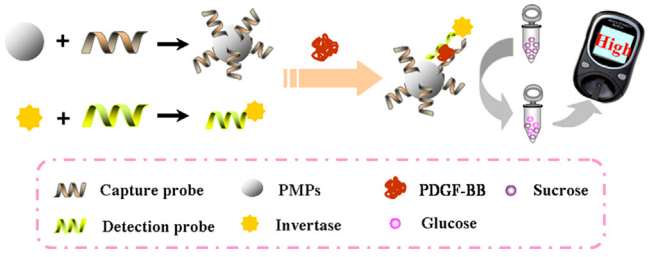

Fig. 1. Schematic of the portable biosensor for PDGF-BB assay using the PGM as readout based on the specific recognition between aptamer and protein.

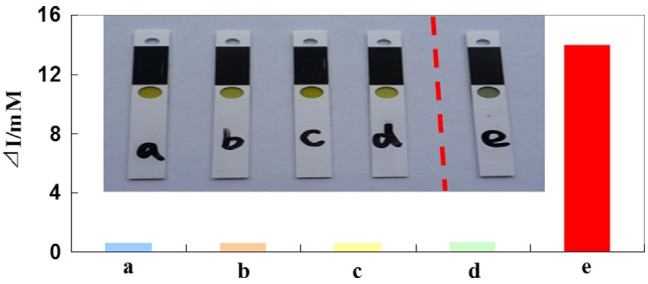

Fig. 2. The enhanced signal of the PGM under the control experiments. The control experiments are the reaction between different mixture and sucrose. (a) The buffer B solution; (b) the solution of SA-PMPs; (c) SA-PMPs modified capture probe; (d) c+invertase-labeled secondary aptamer; and (e) d+PDGF-BB. Inset in Fig. 2: the corresponding color change of the round control window on the back of the test strip in the control experiments. 1.0 M sucrose solution has been added to a different mixture and reacted for 2 h before testing.

to the modification of invertase on the surfaces of SA-PMPs. And invertase could catalyze the hydrolysis of sucrose into glucose and fructose (Paine et al., 1925), resulting in a much higher signal detected by the PGM. The change in the PGM values has a relationship with the PDGF-BB concentration, and a sensitive biosensor for PDGF-BB determination based on this principle can be developed.

Control experiments have been performed to verify the feasibility of the proposed mechanism. The results were summarized in Fig. 2 for buffer B solution (as the blank) (histogram a), SA-PMPs (histogram b), SA-PMPs modified capture probe (histogram c) and the mixed solution of SA-PMPs and invertase-labeled secondary aptamer (histogram d); the signals from the PGM were low, which indicated that no obvious amount of glucose was generated. This also indicates that the proposed method has a lower background signal. On the contrary, a much higher signal had been detected (histogram e) if the PDGF-BB had been added into the reaction system at condition d, displaying that the sucrose has been converted into glucose by the invertase. This result also demonstrates that the invertase on the surface of SA-PMPs still preserves its catalytic activity. In addition, the result of PGM can be estimated by the test strip via the round colored control window on the back itself. In the above mentioned four control experiments, the sucrose cannot be converted into glucose; thus the color does not change (yellow, strips a, b, c and d in the inset). While in the presence of PDGF-BB, sucrose has been converted to glucose by the invertase, which results in color change (green, strip e in the inset). The enhanced PGM signal has some relationship with the concentration of PDGF-BB, which can be applied for quantitative analysis. Therefore, using the PGM for the portable biosensor is feasible.

3.2. Optimization of the experimental conditions

In order to acquire better assay results, the time of specific recognition between two aptamers and PGDF-BB had been optimized firstly. As shown in Fig. 3A, with the increase of the reaction time, the detected signals increased sharply at first, then reached a plateau after 1.5 h. This indicates that 1.5 h is enough for the combination of the two aptamers and PDGF, which has been chosen as the optimized condition in the following study.

Furthermore, the effect of enzyme catalysis time on the readout signals has also been studied. The signal of the PGM increases with the increase of the reaction times (Fig. 3B). The reason lies in that more glucose had been produced at prolong reaction times. Considering that the detection limit at 20 min is low enough for our system, 20 min had been chosen in this study.

3.3. Performance of the biosensor

The signals of the PGM at various concentrations of target under the above optimized conditions are monitored. As shown in Fig. 4A, with the increase of PGDF-BB concentration, the enhanced signal of the PGM increases gradually. The enhanced signal has a good linear relationship with the logarithmic concentration of PGDF-BB in the range of 1.0×10^{-14} M$\sim$$3.16\times10^{-12}$ M (see the inset in Fig. 4A), and the regression equation can be expressed as follows:

$$\Delta I = 4.953 + 3.740 \lg C, \quad R = 0.9965$$

where C is the concentration of PGDF-BB, ΔI is the changing value of the PGM in the presence and absence of PGDF-BB, and R is the regression coefficient. The detection limit is calculated to be 2.9 fM (defined as $S/N=3$). This detection limit is much lower than the earlier reported aptamer-based colorimetric assay (6 nmol/L) (Chang et al., 2013) and electrochemical biosensor (63 pmol/L) (Wu et al., 2010). Furthermore, in addition to the signal of the PGM, the colors from the control window changes gradually from yellow to green along with the increasing concentration of PGDF-BB (see Fig. 4B.

Five parallel repeated experiments had been performed when the concentration of PGDF-BB was settled as 1.0×10^{-13} M, and the relative standard deviation (RSD) was calculated to be 3.1%. These results indicate that the proposed method has good reproducibility. In addition, after being stored in a refrigerator at 4 °C for seven weeks, each week some capture probe and detection probe were took out for target assaying; the signal detected has no significant difference with that obtained by the freshly prepared one.

3.4. Selectivity of the biosensor

Several proteins (such as lysozyme, human serum albumin (HAS) and thrombin) were used as interferents to investigate the selectivity of the portable biosensor. The concentration of interferents was settled as 1.0×10^{-10} M and the target concentration was 1.0×10^{-12} M. The enhanced signal of the PGM and the color of the round colored control window were recorded simultaneously. As shown in Fig. 5, almost no color change in the round colored control window in the presence of interferents had been observed. At the same time, low values were detected by the PGM. On the contrary, the signal of the PGM has a high value and the color of the control window changes from yellow to green obviously in the presence of target. These phenomena indicate that interference from others can be ignored and the proposed strategy shows excellent selectivity for PGDF-BB detection.

3.5. Analytical application

To validate the application of this method in the real sample, three clinical human saliva samples (made available by the first affiliated hospital of Fujian Medical University, China) were tested. The clinical human saliva samples were diluted with Milli-Q reagent water in a 1:10 ratio before testing. Different PGDF-BB standard solutions were spiked into the human saliva samples; the results are shown in Table 1. The recoveries of the PGDF-BB were in the range of 96.5–102%. These results illustrated that the proposed sensor could be applied effectively to distinguish PGDF-BB in complex biological samples with a satisfactory accuracy.

4. Conclusion

In summary, a sensitive and portable biosensor for PGDF-BB detection in complex matrices based on the specific recognition between aptamer and protein has been successfully developed. With the addition of PGDF-BB into the biosensor to initate the

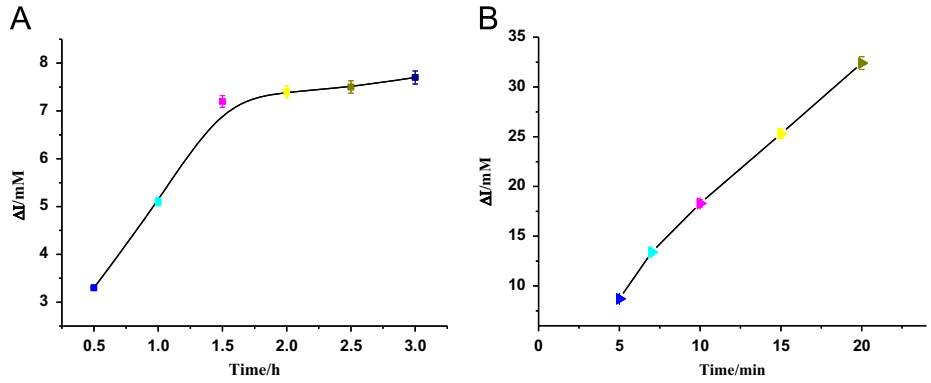

Fig. 3. (A) The effect of the reaction time between two probes and PGDF-BB on the enhanced signal of the PGM and (B) the effect of the operation time between invertase and sucrose on the enhanced signal of the PGM. The concentration of the added sucrose is 1.0 M.

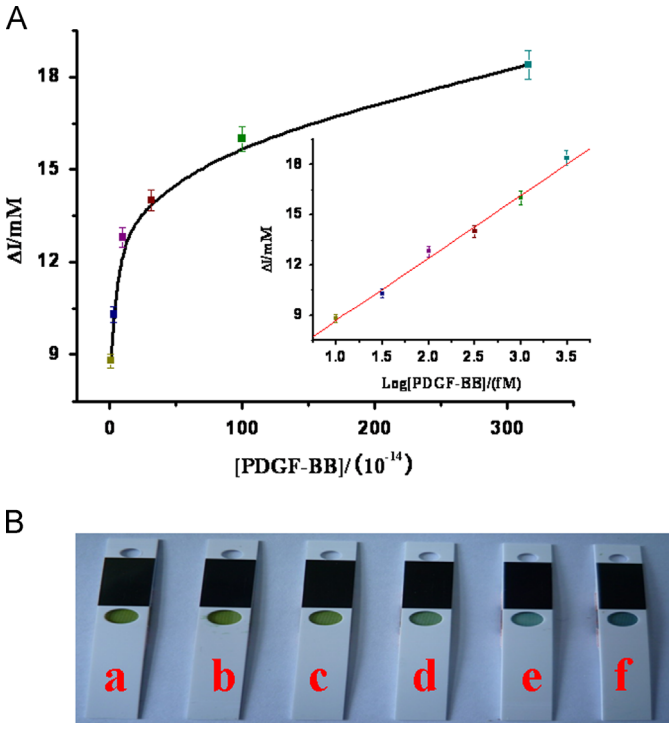

Fig. 4. (A) The relationship between the enhanced signal of the PGM and the PGDF-BB concentration and (B) the color change of the round control window on the back of the test strips at different PGDF-BB concentrations. From a to f, the concentrations of PGDF-BB were 1.0×10^{-14} M, 3.16×10^{-14} M, 1.0×10^{-13} M, 3.16×10^{-13} M, 1.0×10^{-12} M and 3.16×10^{-12} M, respectively. The inset figures in (A) show a calibration curve between the logarithm of PGDF-BB concentrations and the enhanced signal of the PGM.

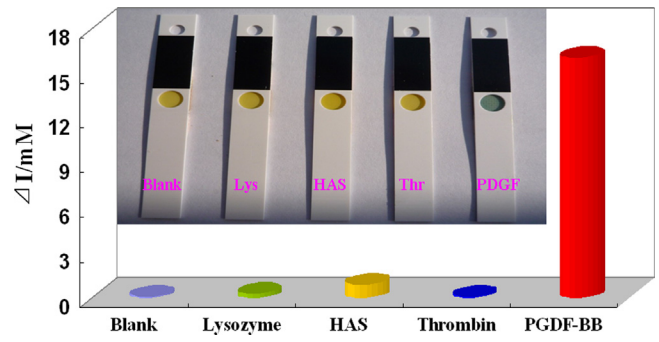

Fig. 5. The enhanced signals from the PGM and the inset: the corresponding color of the round control window at different interferents and targets. Concentrations of lysozyme, HAS and thrombin were 1.0×10^{-10} M, and that of PGDF-BB was 1.0×10^{-12} M. The concentration of each probe is 4 μM.

Table 1
Determination of PGDF-BB in the clinical saliva specimen using the proposed sensor[a].

Sample number	Added (M)	Found (M)	Recovery (%)	RSD (%) ($n=5$)
1	1.00×10^{-12}	1.02×10^{-12}	102	2.9
2	2.00×10^{-13}	1.93×10^{-13}	96.5	3.8
3	5.00×10^{-14}	4.98×10^{-14}	99.6	3.1

[a] The clinical human saliva specimen was diluted 10 times before determination.

combination of aptamer and protein, invertase-labeled SA-PMPs can convert sucrose into glucose, resulting in the increasing signal of the PGM and accompanied with the change of color of the control window from yellow to green. The quantification of PGDF-BB is relevant to the enhanced signal of the PGM and the change of color in test strips, and 2.9 fM of PGDF-BB can be analyzed by this strategy. In addition, the biosensor had also been successfully applied to detect PGDF-BB in biological samples with satisfactory results, such as clinical human saliva samples. The presented sensor provides a reliable option to determine PGDF-BB with high sensitivity and good selectivity. Therefore, given its pocket-size, easy operation, low-cost and wide availability, the proposed portable biosensor may have wide potential applications in clinical biological analysis and may lead to determination of rapid, online, real time, even employed in personal families.

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