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Colorimetric and Ultrasensitive Bioassay Based on a Dual-Amplification System Using Aptamer and DNAzyme

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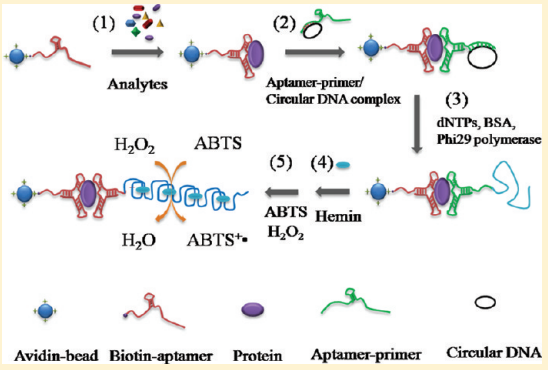
Supporting Information

ABSTRACT: Rapid detection of ultralow amount of biomarkers in a biologically complex mixture remains a major challenge. Herein, we report a novel aptamer-based protein detection assay that integrates two signal amplification processes, namely, polymerase-mediated rolling-circle amplification (RCA) and DNA enzyme-catalyzed colorimetric reaction. The target biomarker is captured in a sandwich assay by primary aptamer-functionalized microbeads (MBs) and a secondary aptamer that is connected to a RCA primer/circular template complex. RCA reaction, which amplifies the single biomarker binding events by a factor of hundreds to thousands (the first amplification) produces a long DNA molecule containing multiple DNAzyme units. The peroxidase-like DNAzyme catalyzes the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (the second amplification), which generates a blue-green colorimetric signal. This new biosensing platform permits the ultrasensitive, label-free, colorimetric detection of biomarker in real time. Using platelet-derived growth factor B-chain (PDGF-BB) as a model system, we demonstrated that our assay can detect a protein marker specifically in a serum-containing medium, at a concentration as low as 0.2 pg/mL in ~2 h, which rivals traditional assays such as ELISA. We anticipate this simple methodology for biomarker detection can find utility in point-of-care applications.



Rapid and sensitive assays for the quantitative detection of biomarkers (i.e., proteins, peptides, lipids, and small molecules) are of tremendous importance in clinical settings for disease diagnosis, proteomics, and metabolomics.[1−4] In particular, many biomarkers that are used to diagnose the early stage of a disease or pathological condition, including cancer, are often present at a very low concentration, which demands ultrasensitive assays.[3,4] In addition, there is an urgent need for simple and rapid point-of-care diagnostics that can be used by nontrained personnel in the developing countries.[5−7] Traditional bioassays, which often use antibodies as biorecognition molecules, include enzyme-linked immunosorbent assay (ELISA),[8] immunomagnetic assay,[9] electrochemical assay,[10] and surface plasma resonance.[11] Although these are well-established and have been widely used, they are complex and time-consuming, often lack sensitivity, and sometimes rely on sophisticated and expensive instruments to process the signal.

Functional nucleic acid (FNA),[12] including DNA aptamers (single-stranded DNA molecules that can specifically bind to a non-nucleic acid target)[13] and DNA enzymes (DNAzymes) (DNA molecules that are capable of catalyzing chemical reactions)[14] represent an emerging class of biomolecules that finds great advantages in bioassay development. Aptamers and DNAzymes for a given target can be isolated using an in vitro method called systematic evolution of ligands by exponential enrichment (SELEX) or in vitro selection.[15,16] Once the sequence is identified, it can be synthesized in a large scale by a DNA synthesizer with almost no batch-to-batch variation and various functional molecules such as biotin (surface anchor) and organic dyes (signaling molecules) can be incorporated at desirable positions without compromising their functions.[17−19] In addition to their high affinity, specificity, and chemical stability at polymerization (e.g., polymerase chain reaction (PCR) and at room temperature, FNA-based assays can be integrated with a variety of established molecular biology tools such as DNA rolling circle amplification (RCA)) and ligation.[20−28] Indeed, the simplicity and versatility have made FNA an emerging candidate in the past decade for bioassay platform development including fluorescent, electrochemical, colorimetric, and others.[12] In particular, the colorimetric FNA assays can be readily applied onto a solid substrate such as cellulose membrane, microfluidic chip and paper which holds great potential in point-of-care applications.[5−7,12]

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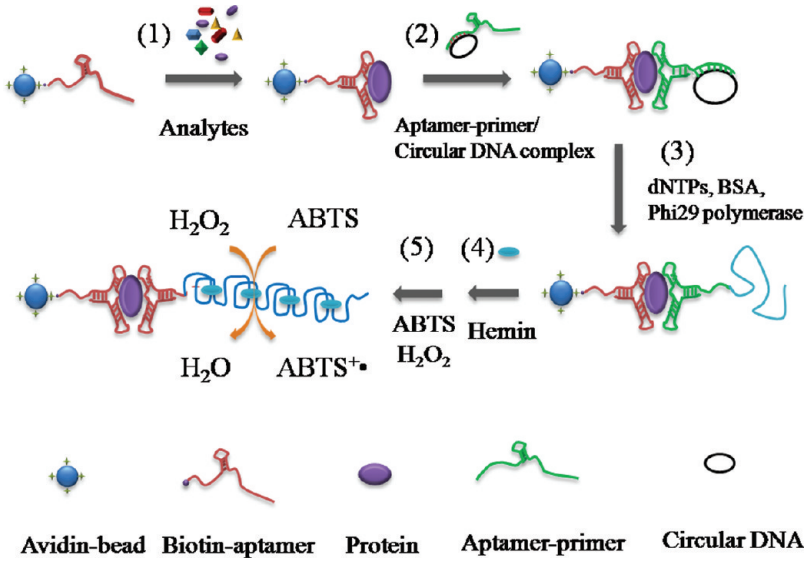
Scheme 1[a]



[a]Schematic representation of the colorimetric assay for protein detection using sandwiched aptamer recognition mechanism and dual signal amplification by RCA and DNAzyme-ABTS reaction: (1) aptamer beads capture the target biomarker in sample solution; (2) the complexes of aptamer-primer/circular DNA templates are subsequently bound to the surface-capture biomarkers on MBs; (3) in the presence of dNTPs and DNA polymerase, rolling circle amplification is initiated and generates single-stranded, long, repetitive DNA chains of DNAzyme domains. After incubated with hemin (4), these multiple DNAzyme domains catalyzed the oxidation of ABTS, producing a blue-green colorimetric signal, which was used as a transduction reporter (5).

Herein, we report a simple colorimetric assay system for protein detection using DNA aptamer coupled to RCA, which generates a large number of peroxidase-like DNAzyme to produce a colorimetric readout signal (see Scheme 1[a]). Specifically, target aptamer coated bead (Aptamer-Bead) is utilized to concentrate target biomarker, which also allows easy removal of excess biomarkers and other unbound materials by washing. In the next step, a secondary aptamer, which is connected to a RCA primer/circular DNA template (Aptamer-primer/Template), binds to the captured biomarker. Subsequently, RCA reaction is performed where DNA polymerase (i.e., phi29) produces a long single-stranded DNA molecule by replicating the circular DNA template hundreds to thousands of times (the first enzyme amplification) under isothermal conditions.[22−28] We purposely designed the circular DNA template with the cDNA sequence of a peroxidase-like DNAzyme that has multiple turnovers (the second enzyme amplification) and can catalyze a chemical reaction to produce color. The DNAzyme is a G-quadruplex moiety that, upon complexing with hemin, mimics the functions of peroxidase.[29,30] The DNAzyme catalyzes the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), generating a blue-green colorimetric signal, which can be monitored simply by the naked eye or a spectrophotometer.[29,30] Here, we used a platelet-derived growth factor B-chain (PDGF-BB), for which aptamers have been developed, as a model target, because PDGF is a cancer-related protein and has been directly implicated in many cell transformation processes and in tumor growth and progression.[18,31]

EXPERIMENTAL SECTION

Reagents and Samples. Recombinant human PDGF-BB was purchased from Bowling Vaccine & Pharmaceutical, Inc. (San Francisco, CA). Bovine serum albumin (BSA), human serum albumin (HSA), human immunoglobulin G (IgG), thrombin, bovine serum were obtained from Dingguo Biotechnology Co., Ltd. (Beijing, PRC). *Escherichia coli* DNA ligase set, Phi29 DNA polymerase and deoxyribonucleoside 5′-triphosphate mixture (dNTPs), *Escherichia coli* Exonuclease I (Exo I), and Exonuclease III (Exo III) were obtained from New England Biolabs (Beijing, PRC). SYBR Green I (supplied as 10 000×) was obtained from Molecular Probes (Beijing, PRC). Avidin-agarose microbead (Avidin bead) from egg whites and 2,2′-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) were purchased from Sigma−Aldrich (Beijing, PRC). H_2O_2, hemin, tris(hydroxymethyl) aminomethane (Tris), trisodium citrate, KH_2PO_4, Na_2HPO_4, and NaCl were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, PRC). All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (electrical resistance of >18.3 MΩ) (Millipore, Billerica, MA).

Oligonucleotides were synthesized from Invitrogen (Beijing, PRC). The thermodynamic parameters of oligonucleotides were calculated using a bioinformatics software (http://www.bioin-fo.rpi.edu/applications/). Biotinylated capture aptamer, 5′-CAG GCT ACG GCA CGT AGA GCA TCA CCA T GA TCC TG TTT TTT-biotin-3′; aptamer-primer probe, 5′-CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TGT TTT TTT CTC ACA CGA ATT CAT CTG AC-3′; padlock probe, 5′-ATT CGT GTG AGA AAA CCC AAC CCG CCC TAC CCA AAA GTC AGA TGA-3′; ligation template, 5′-p CTC ACA CGA ATT CAT CTG AC-3′ (*p* = 5′-phosphate); aptamer-DNAzyme, 5′-CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TGT TTTTTT AAT TCA TCT GAC TTT TGG GTA GGG CGG GTT GGG TTT TCT CAC ACG-3′.

Preparation of PDGF-BB Aptamer/Bead Conjugates. Avidin beads were first washed using PBS solution (10 mM phosphate buffer, pH 7.2, 100 mM NaCl) three times by centrifugation and finally resuspended in PBS at a final

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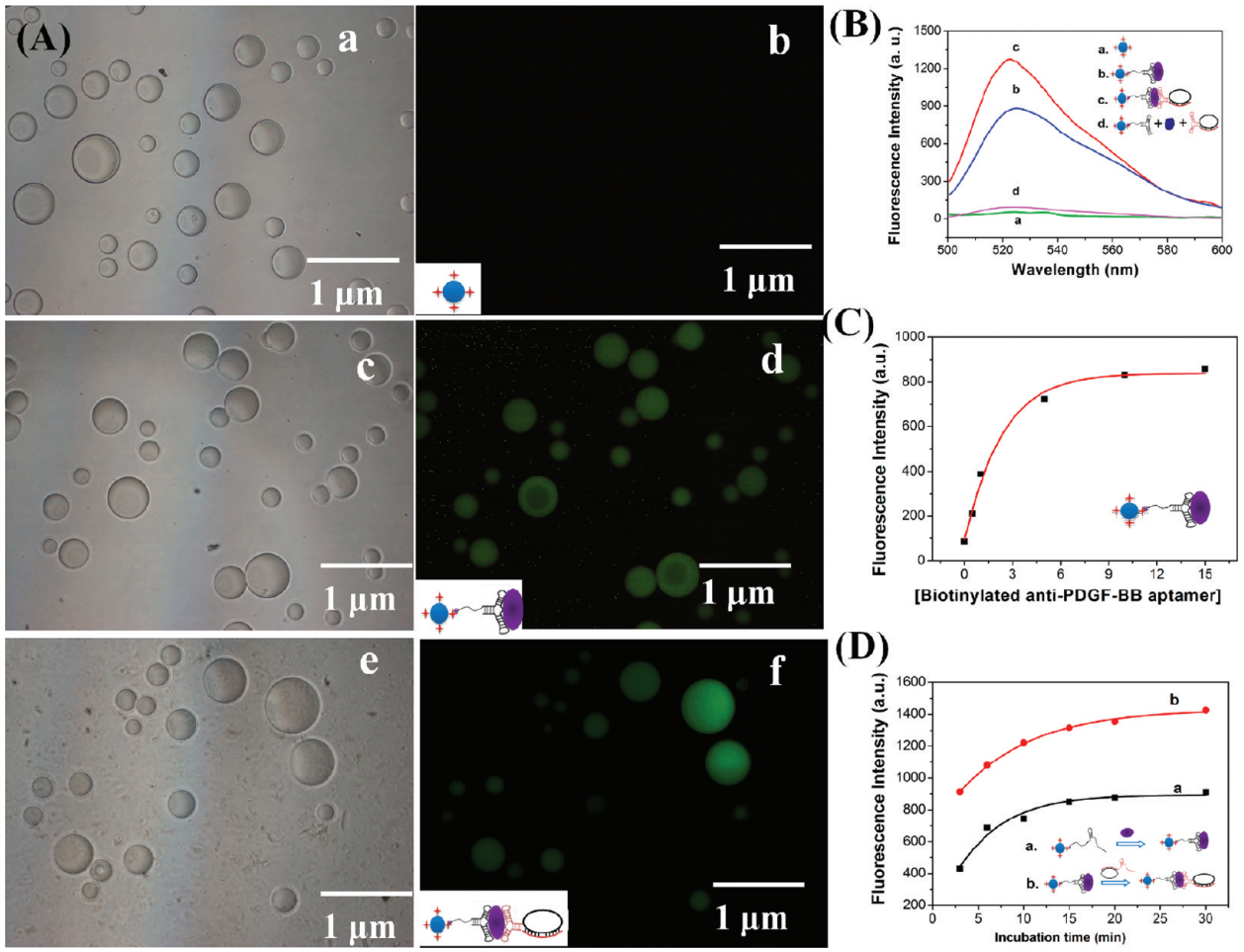

Figure 1. Fluorescence monitoring of conjugated primary aptamer, PDGF-BB, secondary aptamer-primer/template onto Avidin beads with SYBR Green I as the fluorescent indicator. (A) Bright field and fluorescence microscopy images of a glass slide drop-coated with beads solution ((a, b) Avidin beads, (c, d) primary aptamer beads with PDGF-BB, (e, f) aptamer beads with PDGF-BB and aptamer-primer/template). (B) Fluorescence emission spectra of SYBR Green I at 520 nm of (a) aptamer beads and subsequently incubated with (b) PDGF-BB and (c) aptamer primer/template (in panel (d), primary aptamer beads with BSA then aptamer primer/template were used as a control). (C) Fluorescence signal of an aptamer–beads complex formed with different concentrations of biotinylated anti-PDGF-BB aptamer ([avidin beads] = 50 μg/mL; [biotinylated PDGF-BB aptamer] = 0, 0.5, 1, 5, 10, 15 μM (from left to right)). (D) Fluorescence signal of aptamer beads (curve a) and aptamer beads/PDGF-BB/aptamer primer/template (b) with different incubation times, ranging from 3 min to 30 min. Excitation and emission wavelength of SYBR Green I were 475 and 520 nm, respectively.

concentration of 10 mg mL^{-1}. To a solution with 500 μL of avidin-MB, 500 μL (20 μM) of biotinylated capture aptamer that binds to PDGF-BB was added and incubated for 15 min at 37 °C. The resulting aptamer beads were washed three times, using 2 mL of PBS, to remove the free capture aptamers. The sediment was collected and resuspended in 0.5 mL of PBS. The resultant aptamer bead solution was stored at 4 °C for the subsequent experiments.

Preparation of the Aptamer Primer/Template. The circular DNA template was prepared as follows.[22−26] First, 2 μL of the linear, phosphorylated padlock DNA (3 mM) and 6 μL ligation template (3 mM) were hybridized in the ligation buffer (300 mM Tris-HCl, pH 8.0, 40 mM $MgCl_2$, 100 mM $(NH_4)_2SO_4$, 12 mM EDTA, and 1 mM NAD^+) at 37 °C for 30 min. Second, 0.5 μL T4 DNA ligase (60 U μL^{-1}), 10 μL 0.05% BSA, 7 μL of 10× ligase buffer, and 48 μL of ultrapure water were added, and the mixture was allowed to incubate overnight at 16 °C. Next, Exo I (1 μL, 20 U μL^{-1}) and Exo III (0.5 μL, 100 U μL^{-1}) were added to digest the leftover ssDNA and dsDNA to yield the circular DNA. The enzymes were denatured by heating at 90 °C for 10 min. The ligated circular DNA was then purified and separated from the ligation reaction solution using a centricon filtration device (10 000 cutoff, Millipore, Inc.), and then dispersed into a 50-μL buffer solution. Finally, the as-synthesized circular DNA and the aptamer primer (2 μL, 1 mM) were annealed in PBS at 55 °C for 2 h and stored at 4 °C until use.

Rolling Circle Amplification. Target protein PDGF-BB in various amounts were added to each of the 5 μL of aptamer beads in a final volume of 100 μL PBS in a PCR tube. The reaction mixture was shaken at 1200 rpm at 37 °C for 15 min. After centrifugation, the beads were washed with 100 μL PBS buffer to remove the nonspecifically bound protein, and then resuspended in 10 μL PBS buffer. Next, the resultant PDGF/aptamer/beads were incubated with the aptamer primer/template solution for various times and the resultant beads were washed by centrifugation using PBS. The RCA reaction was subsequently conducted in a 50-μL solution that contained 50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 50 nM primer/circular template (on bead), 1 mM dNTP, 0.1 mg/mL

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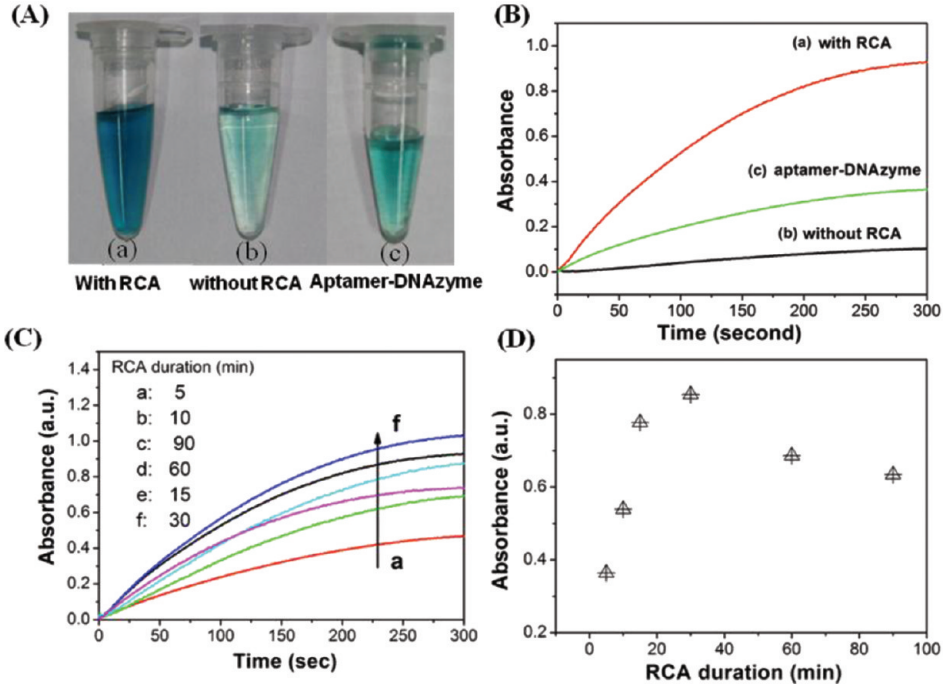

Figure 2. (A) Representative photograph and (B) UV−vis spectra at 415 nm in the DNAzyme-mediated ABTS-H_2O_2 system ((a) PDGF-BB/aptamer/beads complexed with aptamer primer/template with subsequent RCA reaction; (b) PDGF-BB/aptamer/beads complexed with aptamer primer/template without subsequent RCA reaction; (c) PDGF-BB/aptamer/beads complexed with a single unit aptamer-DNAzyme, used as a control). (C, D) Absorbance measurement of the ABTS oxidation catalyzed by the DNAzyme generated in 5−90 min RCA reaction. In Figure 2C, the time on the *x*-axis is the DNAzyme assay time for a given RCA product. The time in plots a−f is the RCA reaction time that is proportional to the length of RCA products. [PDGF-BB] = 1 ng/mL; [hemin] = 1.0 μM; [ABTS] = 3.2 μM; [H_2O_2] = 2.4 mM. In this figure, the absorbance was measured in solution-containing beads.

BSA, 1 mM DTT, and 5 units of phi29 DNA polymerase. The reaction mixture was incubated at 30 °C for 10−90 min and terminated by heating at 65 °C for 10 min. Ten microliters (10 μL) of freshly made hemin solution (5 mM) was then added to the RCA product. The mixture was incubated in room temperature for 1 h to allow the hemin to complex with DNA.

Colorimetric/Absorbance Measurement. For the colorimetric measurement, the ABTS/H_2O_2 substrate (1.17 mM and 2.82 mM, respectively) was added to the RCA product solution to yield a total volume of 100 μL. The beads-containing reaction solution was characterized by UV−vis absorption spectra, which were recorded using a Beckman Model Du520 general purpose UV/vis spectrophotometer. Kinetic data were obtained by recording the absorbance at 415 nm every 5 s during the first 5 min of the reaction.

Fluorescence Microscopy Imaging. Fluorescence microscopy images were taken using a Nikon TE-300 inverted optical microscope integrated with a Cohu 4912 CCD camera.

RESULTS AND DISCUSSION

In our strategy, the aptamer beads are used to capture the target protein (PDGF-BB) and then bind to a secondary aptamer that is conjugated with a RCA primer (see Scheme 1[a]). First, the binding of PDGF-BB to aptamer beads was investigated. The specific recognition process was demonstrated using fluorescence microscopy images based on fluorescence staining of intramolecular structure in PDGF aptamers.[31,32] It is known that, in the presence of PDGF, the PDGF aptamer folds into a hairpin structure, which can be stained effectively by SYBR Green I.[16,31,32] Indeed, a strong green fluorescence was observed when the aptamer beads were incubated with PDGF-BB (see Figure 1A (c and d)). In contrast, no fluorescence was observed with control Avidin-Beads after stained by SYBR Green I (Figure 1A (subpanels a and b)). Moreover, after further incubating with aptamer primer/template complex, the PDGF/aptamer/beads showed a much stronger fluorescence signal (Figure 1A, subpanels e and f), indicating that the RCA primer/template complex was successfully captured on the beads. By contrast, when a nontargeting protein (BSA) was added at the same concentration, no significant fluorescence signal was observed (see Figure S1 in the Supporting Information), indicating that the binding event between the aptamer and the target protein is specific. The fluorescence spectra, which are more quantitative, were consistent with the microscopy images (see Figure 1B). By contrast, in control experiments where no protein (Figure 1B) or 1 μM BSA (data not shown) were added, little fluorescence signal was observed.

We further optimized the concentration of primary aptamer used for immobilization on avidin beads and the incubation time between aptamer and target protein in order to minimize the use of reagents and reduce the assay time (see Figure 1C). Using SYBR Green I as a signal indicator as established above, we monitored the fluorescence of primary aptamer at different concentrations when incubated for 30 min in the presence of a fixed concentration of avidin beads (50 μg/mL) and PDGF-BB (1 ng/mL). It is demonstrated that the fluorescence peak intensity increased rapidly with the increase of aptamer concentration and then reached a plateau after ~10 μM. Therefore, we used aptamer of 10 μM for 50 μg/mL avidin beads for the subsequent experiments. We then optimized the incubation time between aptamer beads and PDGF-BB (Figure

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1D, line a) and between PDGF/aptamer/bead and secondary aptamer primer/template complex (Figure 1D, line b). Both of the fluorescence peak intensity increased proportionally with the incubation time and started to reach a plateau at ~15 min. Therefore, we chose an incubation time of 15 min for both binding events in the subsequent experiments. Initially, we were concerned that the MBs and large molecules might influence the ultraviolet−visible light (UV-vis) detection, because of the light scattering phenomenon. Interestingly, we did not observe any significant interference from scattering to our actual fluorescence signal. We attributed this to the low concentration of MBs. In addition, we did not observe any significant aggregation either when PDGF-BB was added into the aptamer-beads solution.

Next, we performed RCA reaction on PDGF-BB captured beads via aptamers in a sandwich format (which was prepared using an initial PDGF concentration of 1 ng/mL) (see the Experimental Section). The successful production of long RCA product was directly evidenced by a >20 fold fluorescence signal increase stained with SYBR Green I after RCA reaction (see Figure S2 in the Supporting Information). As we stated above, we have encoded the complementary sequence of a peroxidase-like, G-rich quadruplex DNAzyme sequence in the RCA circular template, and therefore, the RCA reaction produces a long DNA molecule that contains multiple units of DNAzymes. The RCA product was characterized by gel electrophoresis (see Figure S3 in the Supporting Information). To assess whether the DNAzyme units in the long RCA product are catalytic, we used the ABTS−H_2O_2 system, where H_2O_2-mediated oxidation of ABTS produces free-radical cation $ABTS^{+}$.[29,30] $ABTS^{+}$ has a blue-green color signal with a maximal absorption at 415 nm, which was used to quantitatively monitor the peroxidase-like activity of the hemin−aptamer complex.[29,30] As shown in Figures 2A and 2B, the RCA product that was complexed with hemin rapidly produced (within several min) a signal at 415 nm or a blue-green color after the addition of ABTS and H_2O_2. In contrast, without RCA reaction, PDGF/aptamer/MB complexed with aptamer-primer/template itself only produced minimal signal. In addition, to demonstrate the importance of RCA reaction in our sensor performance (i.e., low detection limit), we directly compared it with PDGF/aptamer/MB complexed with a single unit aptamer-DNAzyme (see Figures 2A and 2B). It clearly demonstrated that RCA product with multiple copies of DNAzyme produced a significantly higher signal than the single-unit DNAzyme.

We next studied the effect of RCA reaction time on the sensing performance. As shown in Figures 2C and 2D, the absorbance at 415 nm first increased with the increase of RCA reaction from 5−30 min but then decreased at longer RCA reaction time (30−90 min). We reasoned that, below 30 min, longer RCA reaction time produced more DNAzyme units, which yielded a higher signal. However, when the reaction time is longer than 30 min, the resultant long RCA products may have significant intramolecular or intermolecular interactions, particularly because this particular DNAzyme sequence has many G-rich segments, which makes the DNAzymes less available and functional.[29,30] Given that there is little signal difference between 20 and 30 min RCA reaction time and that we want to minimize the assay time, we chose 20 min RCA reaction time for the subsequent experiments.

We next demonstrated that the color signal produced by our system was dependent upon PDGF-BB concentration ranging from 1 pg/mL to 1000 pg/mL (Figure 3). As the PDGF-BB concentration increases, the absorbance at 415 nm increases

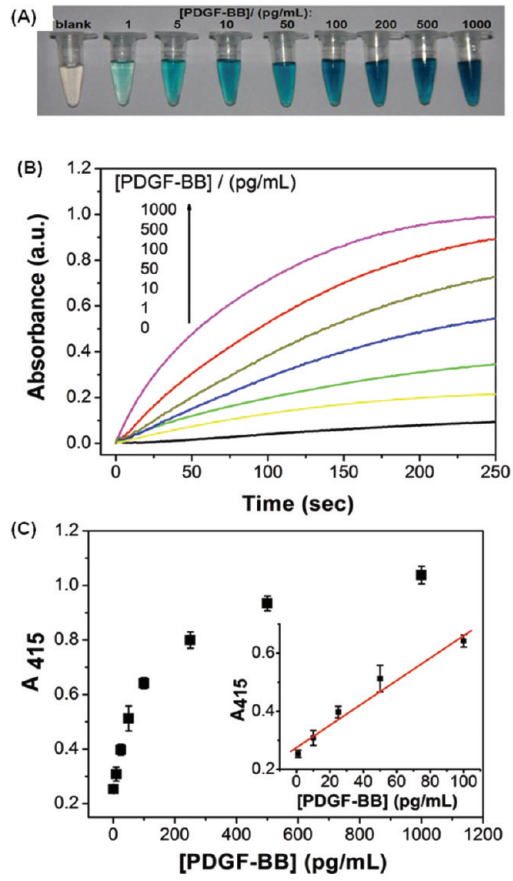

Figure 3. Colorimetric PDGF-BB analysis using the dual-amplification assay based on aptamer and DNAzyme. (A) Representative photograph of PDGF-BB detection using RCA-produced DNAzyme mediated ABTS-H_2O_2 system. The photos were taken after 5 min oxidation of ABTS catalyzed by the DNAzymes. (B) The time-dependent absorbance changes upon analyzing different concentrations of target protein (PDGF-BB). (C) Dependence of absorbance at 415 nm (A415) on PDGF-BB concentration. Inset shows a linear range from 1 pg/mL to 0.1 ng/mL. RCA reaction time = 20 min; DNAzyme assay time = 3 min.

correspondingly. A quasi-linear correlation was obtained in the concentration ranging from 1 pg/mL to 100 pg/mL. According to the definition that the detection limit is the lowest analyte concentration required to produce a signal greater than 3 times the standard deviation of the noise level (3α), the limit of detection (LOD) for this system is 0.2 pg/mL (~7.1 fM, given the molecular weight of PDGF-BB is ~28 000). Our assay system demonstrates sensitivity that is comparable to or even greater than that of the ELISA assay, which typically detects analytes from picomolals to nanomolals.[33,34] With comparison to those aptamer-based sensing systems, which integrated with colorimetric, luminescent, fluorescent, and electrochemical techniques (Table 1),[25,26,35−40] our system represents a 1- to 1000-fold improvement in term of sensitivity, together with a wide linear dynamic range. Furthermore, the detection sensitivity and LOD could be further improved in the future work using magnetic beads where magnetic beads captured with target analytes can be readily concentrated by a magnet. In addition, the entire assay procedure requires ~2 h, which is

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Table 1. Summary of Recent PDGF-BB Assays Using Aptamer as a Recognition Molecule

assay	indicator or amplification	linearity range	detection limit	ref
Electrochemical Assay	Methylene Blue, RCA	84 pM−8.4 nM	63 pM (1.8 ng/mL)	25
	RCA and silver deposition	10 fM−100 pM	10 fM (0.28 pg/mL)	26
	ferrocene, proximity-dependent surface hybridization assay	1.0 pg/mL−20 ng/mL	36 fM (1.0 pg/mL)	35
colorimetric assay	Au NPs	15 nM−100 nM	3.2 nM (90 ng/mL)	36
fluorescence assay	isothermal polymerization amplification	25 ng/mL−1.0 μg/mL	0.43 nM (12 ng/mL)	37
	fluorescent ferritin nanoparticles		100 fM (2.8 pg/mL)	38
lumilescence	$[Ru(phen)_2(dppz)]^{2+}$	0−50 nM	1 nM (28 ng/mL)	39
capillary electrophoresis		0.5−50 nM	50 pM (1.4 ng/mL)	40

comparable to or rivals current bioanalytical methods such as ELISA and PLA (proximity ligation assay).[32]

To demonstrate the selectivity, control experiments were performed using other proteins or matrices, including immunoglobulins A (IgA, 100 μg/mL), immunoglobulins G (IgG, 100 μg/mL), bovine serum albumin (BSA, 100 μg/mL), α-fetoprotein (AFP, 10 ng/mL), thrombin (10 ng/mL), and glucose oxidase (GOx, 1 μg/mL). As shown in Figure 4A, these

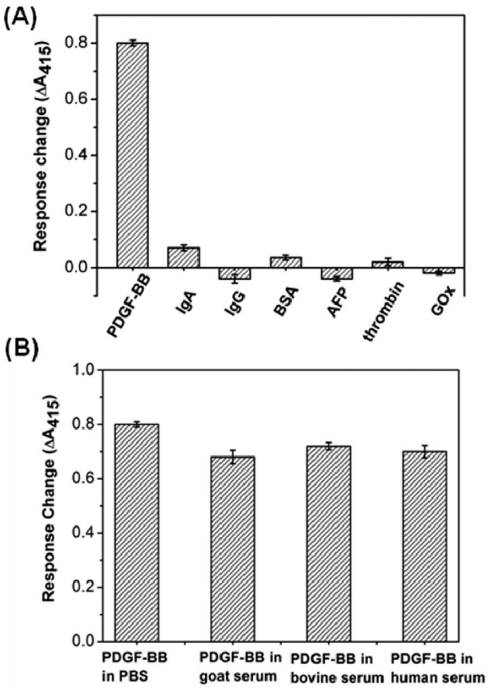

Figure 4. (A) Specificity analysis of the dual-amplification assay based on aptamer and DNAzyme for the PDGF-BB detection in the ABTS-H_2O_2 system. The concentrations of the PDGF-BB, IgA, IgG, BSA, AFP, thrombin and glucose oxidase (GOx) are 1 ng/mL, 100 μg/mL, 100 μg/mL, 100 μg/mL, 10 ng/mL, 10 ng/mL and 1 μg/mL, respectively. (B) Detection of PDGF-BB in complex biological fluid in serum using the sandwich aptamer−dual-amplification assay in the ABTS-H_2O_2 system. One ng/mL PDGF-BB was dissolved in 10% goat, bovine, and human serum samples, respectively.

control groups exhibited minimal signal which is close to the nontarget background. We also spiked PDGF-BB proteins in 10% goat, bovine and human serum samples, respectively, and then determined the concentrations of the proteins in the spiked samples (Figure 4B). The recoveries ranged from 90% to 95%, indicating that our assay possesses a great selectivity and anti-interference capability, even in complex media, and may therefore find practical utility in clinical settings.

CONCLUSION

In summary, we reported a facile colorimetric assay using aptamers as recognition agent in a sandwich format and a dual enzyme processes to significantly amplify the signal. The approach enables rapid biomarker detection with high specificity, low limit of detection (LOD), and short assay time, which may therefore serve as a useful alternative or supplement to conventional assays that typically suffer from complexity and poor sensitivity. In particular, as the aptamers for a particular target can be obtained by SELEX,[15,16] our approach can be widely applied to detect many other targets, including cells. Note that the divalent form of PDGF-BB made it convenient for us to test our concept. However, different aptamer sequences that bind to different regions of the same protein target can be obtained by SELEX. For instance, there are two different aptamers were selected to bind to two different domains of thrombin which is not a dimer.[41] This versatility allows our technology to be widely applied to detect many protein targets. The colorimetric nature and their chemical stability of aptamers and DNAzymes make our assay particularly useful for point-of-care applications in the developing world. While protein enzymes have been widely used in point-of-care devices (e.g., glucose test strips), the use of DNA polymerase in our system may present some challenge when using it in developing countries in terms of cost and storage. We plan in the future to integrate our system with nucleic acid based, protein enzyme-free DNA amplification methods which are more cost-effective and stable in long-term storage.[42]

ASSOCIATED CONTENT

Supporting Information

Gel and fluorescence characterization of RCA product and summary of recent PDGF-BB assays using aptamer as recognition molecule. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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