Anal. Chem. **2007,** *79,* 229−233

Aptamer-Based Electrochemical Detection of Picomolar Platelet-Derived Growth Factor Directly in Blood Serum

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We report an electrochemical, aptamer-based (E-AB) sensor for the detection of platelet-derived growth factor (PDGF) directly in blood serum. The E-AB approach employs alternating current voltammetry to monitor target-induced folding in a methylene blue-modified, PDGF-binding aptamer. The sensor is sensitive, highly selective, and essentially reagentless: we readily detect the BB variant of PDGF at 1 nM directly in undiluted, unmodified blood serum and at 50 pM (1.25 ng/mL) in serum-diluted 2-fold with aqueous buffer. The sensitivity and selectivity achieved by this sensor match or significantly exceed those of the best analogous optical approaches. For example, the detection limit attained in 50% serum is achieved against a >25 million-fold excess of contaminating blood proteins and represents a 4 order of magnitude improvement over the most sensitive optical PDGF aptasensor reported to date. Moreover, the E-AB sensor combines these promising attributes in a platform that is reusable, label-free, and electronic. Given these advantages, E-AB sensors appear well suited for implementation in portable microdevices directed at the direct detection of proteins and small molecules in complex, largely unprocessed clinical samples.

Aptamers, artificial oligonucleotides selected in vitro for their ability to bind to proteins,[1,2] small molecules,[3] and even whole cells,[4] recognize their targets with affinities and specificities often matching or exceeding those of antibodies.[5] As a result, aptamers have been employed as recognition elements in sensors aimed at a wide range of targets and employing an almost equally wide range of mechanical,[6,7] optical[8,9] or electronic[10,11] readout mechanisms.

Aptamer beacons, which are optical sensors based on the binding-induced folding of unfolded or partially folded aptamers, appear to be one of the more promising "aptasensor" approaches.[12,13] Because aptamer beacons couple signal generation to a binding-specific change in the aptamers—rather than to adsorption to the sensor surface—they are relatively immune to false positives arising from nonspecific interactions with contaminants.[13] Nevertheless, aptamer beacons suffer from potential drawbacks that can hinder optical approaches. These include false signals arising from contaminating fluorophores or chromophores, masking by optically dense contaminants, photobleaching, and the operational inconvenience often associated with light sources, optics, and optical detectors. In contrast, the low electroactive background observed in clinical samples, the relative stability of electroactive labels, and the promising speed, cost, and convenience of microelectronics suggest that electrochemical aptasensors might avoid many of these potential pitfalls. Thus motivated, we[14−16] and others,[17] have previously developed electrochemical, aptamer-based (E-AB) sensors, which are the electrochemical analogue of aptamer beacons in that they employ electrochemistry,

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10.1021/ac061592s CCC: $37.00
Published on Web 11/30/2006

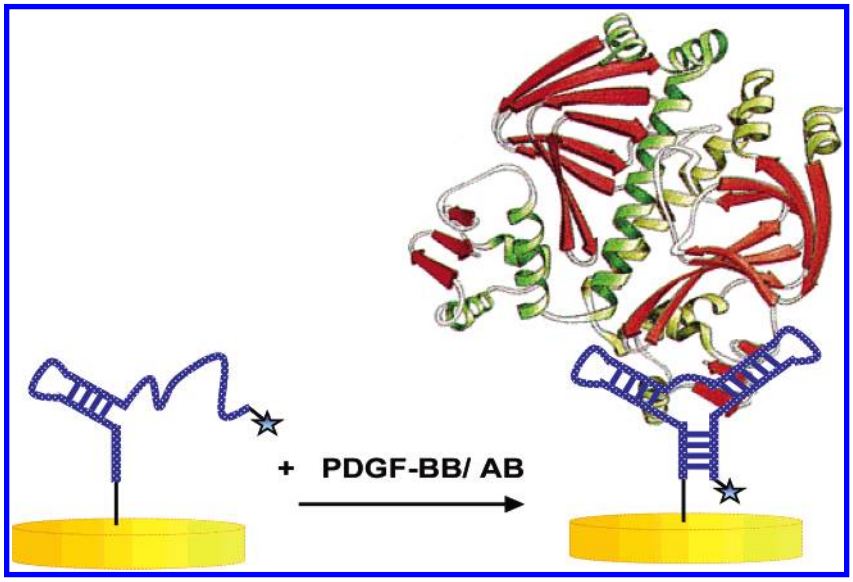

Figure 1. E-AB-based PDGF sensor fabricated by self-assembly of an MB-labeled aptamer on a gold electrode surface. In the absence of target (left), the aptamer is thought to be highly dynamic and partially unfolded, with only one of the three stems intact. Upon target binding (right), electron transfer is more efficient, presumably because the aptamer forms a stable three-way junction holding the MB label close to the electrode surface.

rather than optics, to monitor binding-induced conformational changes (Figure 1) To date, however, no direct comparison of the sensitivity and selectivity of optical and electrochemical aptamer-based sensor approaches has been performed. We report here the fabrication and characterization of an E-AB sensor directed against the blood protein platelet-derived growth factor (PDGF) and its comparison with several previously reported optical techniques directed against the same target and employing the same aptamer.

EXPERIMENTAL SECTION

Iron-supplemented fetal calf-serum, reagent grade hydrogen peroxide (30%), sulfuric acid, 6-mercapto-1-hexanol (C6-OH), sodium dodecyl sulfate, hydrochloric acid, magnesium chloride (all from Sigma-Aldrich, St. Louis, MO), potassium phosphate, and sodium chloride (Fisher Scientific, Fairlawn, NJ) were used as received. Recombinant human PDGF-BB, PDGF-AB, and PDGF-AA (R&D Systems, Minneapolis, MN) were dissolved in 4 mM HCl prior to use. Thiol- and methylene blue (MB)-modified oligonucleotides were obtained commercially (Biosource, Foster City, CA) using *N*-hydroxysuccinimide ester of MB (EMP Biotech).

The PDGF-binding aptamer we have employed, oligomer 1, was adopted from the literature:[13]

5′-HS-$(CH_2)_6$-CAGGCTACGGCACGTAGAGCATCAC-
CATGATCCTG-$(CH_2)_7$-NH_2-MB-3′

A nonbinding control sequence, oligomer 2, was similarly adopted from the literature:[13]

5′-HS-$(CH_2)_6$-CAGCGTACGGCACGTACCGATTCAC-
CATGAAGCTG-$(CH_2)_7$-NH_2-MB-3′

A second control sequence, oligomer 3, which adopts a stem−loop structure not known to bind PDGF, was based on previous studies in our laboratory:[18]

5′-HS-$(CH_2)_6$-GCAGTAACAAGAATAAAACGCCACTGC-
$(CH_2)_7$-NH2-MB- 3′

Electrochemical measurements were performed at room temperature using a CHI 730B Electrochemical Workstation (CH Instruments, Austin, TX), and gold working electrodes (0.88 mm^2) were fabricated on a glass plate using standard microfabrication techniques.[19] The patterned electrodes were cleaned by immersing in piranha solution (3:1 H_2SO_4/H_2O_2) for 5 min and then thoroughly rinsing in deionized water. A platinum wire was used as the counter electrode. All electrochemical potentials are reported versus a Ag/AgCl (3 M KCl) reference electrode.

To fabricate the E-AB sensor, the relevant oligonucleotide was dissolved at 0.2 μM in PBS plus 5 mM $MgCl_2$. A cleaned electrode was immersed in this solution for ∼15 min and then incubated in 2 mM C6-OH in PBS for ∼3 h to displace any nonspecifically bound material. Aptamer coverage of $\sim 2 \times 10^{-13}$ mol/cm^2 is readily obtained with this immobilization method. The surface density was determined using a previously established method utilizing the peak current in the alternating current voltammogram.[20] For these calculations we assume perfect electron-transfer efficiency from the electrode to the redox label. As previously reported for other E-AB sensors,[14] the observed signal change upon target binding is dependent on surface coverage (Supporting Information S.I.1) and is optimal at this surface coverage (data not shown).

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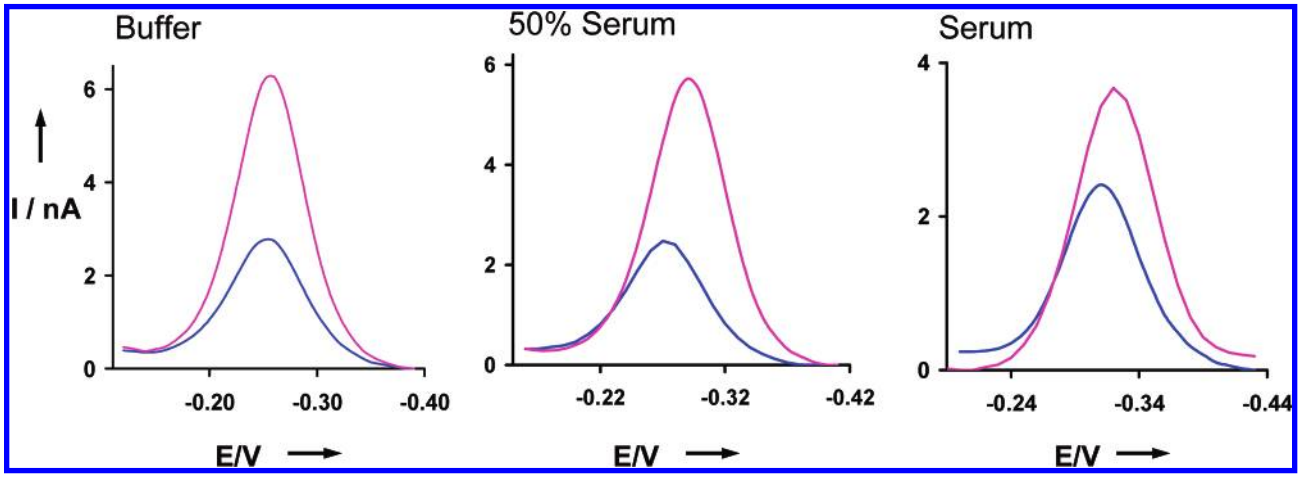

Figure 2. E-AB sensor response in (left) buffer, (middle) 50% serum, and (right) undiluted serum. Shown are baseline-subtracted ac voltammograms for the E-AB sensor before (blue curve) and after (pink curve) ~30-min incubation with 50 nM PDGF-BB. The reduced currents observed with the undiluted serum represent sensor-to-sensor variability (while the relative signal change is highly reproducible for a given surface coverage—see Supporting Information S.I.1—the absolute current depends on surface coverage and the total, microscopic surface area of the electrode). Studies with single electrodes indicate that the reduced signal gain observed in serum is due to an increase in the magnitude of the background current observed in the absence of target (see Supporting Information S.I.2)

Aptamer-modified electrodes were analyzed using alternating current voltammetry over the range −0.15 to −0.43 V versus Ag/AgCl with a 10-Hz, 25-mV ac potential. The electrolytes employed were 500 μL of either 10 mM, pH 7 phosphate buffer with 100 mM NaCl (PBS), undiluted, unmodified fetal calf serum, or serum diluted with PBS. Prior to interrogation, the electrodes were incubated for ~30 min in the relevant electrolyte lacking exogenously added target. The target was added only when the sensor had fully equilibrated/stabilized as determined by stable peak currents. The sensor was then allowed to incuabate in the presence of the target for ~30 min before voltammetric measurements were conducted directly in the sample. Sensor regeneration was achieved via immersing in 10% SDS for 4 min followed by copious rinsing with deionized water. Regeneration was verified by ACV collected after 30-min immersion in PDGF-free electrolyte. We typically employ each electrode as many as five times before significant degradation is observed (data not shown).

RESULTS AND DISCUSSION

The E-AB sensor is constructed by attaching an MB-modified, PDGF-binding aptamer (oligo 1) to a gold electrode via self-assembled monolayer chemistry.[21] In the absence of its target, the aptamer is thought to retain only one of its three double-stranded stems intact,[22] which should have the effect of reducing electron transfer between the electrode and the MB. Upon target binding, the aptamer is thought to fold into a configuration[21] that forces the MB label into proximity with the electrode (Figure 1), leading to improved electron-transfer efficiency.

The E-AB sensor is responsive to its target, PDGF, even in the face of orders of magnitude higher concentrations of contaminants. For example, when the sensor is deployed in pure buffer, we observe a defined reduction peak at −0.25 V (vs Ag/AgCl), characteristic of MB reduction, which shifts by ~0.02 V (presumably due to surface crowding) and increases by 135% in the presence of 50 nM (1.25 μg/mL) PDGF-BB (Figure 2, left). Moreover, because of the specificity of binding-induced folding and the relative paucity of electroactive contaminants, the E-AB sensor can also be employed directly in complex, contaminant-ridden samples. For example, when deployed in 50% serum (diluted 1:1 with buffer), the signal gain of the E-AB sensor is effectively indistinguishable from that obtained in pure buffer (Figure 2, middle) despite the presence of ~65 mg/mL contaminating serum proteins.[23] Finally, the sensor can also be deployed directly in undiluted blood serum. Under these conditions, however, we observe a smaller, 56% increase in the E-AB signal when the sensor is challenged with 50 nM PDGF-BB (Figure 2, right). This reduced signal gain occurs in part due to slow sensor equilibration when deployed in undiluted serum (see below). A perhaps larger contributor to the reduced signal gain, however, is a 50% increase in the initial peak currents obtained under these conditions relative to those observed in buffer or 50% serum (Supporting Information S.I.2). Previous research has suggested that the confirmation of the PDGF-binding aptamer is strongly dependent on the concentrations of divalent cations such as Mg^{2+} and Ca^{2+}.[24] It is thus possible that the effective ion concentration is high enough in undiluted serum to induce significant, additional folding of the aptamer even in the absence of target. Also of note, in undiluted serum, the peak potential moves to −0.30 V (vs Ag/AgCl), presumably due to the slightly alkaline pH of serum and the strong pH dependence of MB reduction.

The sensitivity, selectivity, and dynamic range of the E-AB sensor are exceptional. Even when deployed in 50% blood serum, the sensor achieves a robust detection limit of 50 pM (1.25 ng/mL) (Figure 3), corresponding to detection against a >25 million-fold excess of contaminating blood proteins. Under these conditions, the dynamic range of the sensor extends from 50 pM to tens of nanomolars (Supporting Information S.I.3), a span that readily covers the 400−700 pM serum PDGF concentrations found

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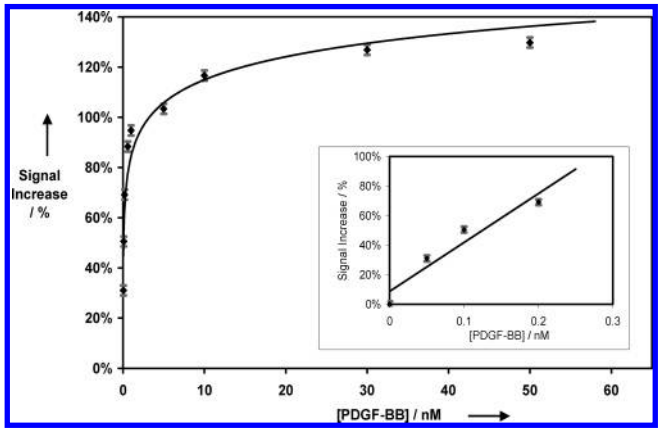

Figure 3. Dose−response curve of the E-AB PDGF-BB sensor in 50% serum. The large signal gain produces a ~30% signal increase at the lowest (50 pM) PDGF concentration investigated. The incubation time was 35 min. The illustrated error bars represent the standard deviation of three measurements obtained at each PDGF concentration. These data were collected with a single electrode; electrode-to-electrode variability is an additional, if reasonably controllable, source of scatter when comparing results obtained using multiple electrodes (see Supporting Information S.I.1).

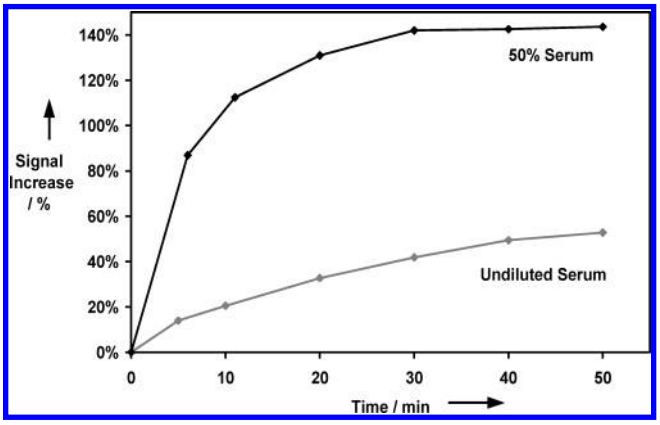

Figure 4. Relatively rapid E-AB sensor. Shown is the typical sensor response after the introduction of 50 nM PDGF-BB in 50% serum and undiluted serum. We presume the slower equilibration time observed in undiluted serum arises due to the significantly increased viscosity of this material.

in healthy individuals and, at the higher end of the range, cancer patients.[25,26] Previous research suggests that the binding of PDGF to this aptamer is biphasic, perhaps due to the existence of two noninterconverting components in the aptamer that bind to the PDGF with differing affinities.[22] Similarly biphasic behavior may also be apparent in our results, as the signal increase with increasing PDGF exhibits small deviations from monophasic behavior between 1 and 10 nM. While slight, this behavior is reproducible and is observed in both diluted and undiluted serum (Figure 3, Supporting Information S.I.3).

The E-AB sensor response is rapid. In 50% serum, we observe complete signal equilibration in less than 30 min, with the signal approximately doubling in the first 10 min (Figure 4). In contrast, the observed binding kinetics in undiluted serum is slower, with signal saturation requiring more than 1 h. We presume the slower response time in undiluted serum arises due to the greater viscosity of this material, which could impede diffusion of the target to the electrode.

Studies of a control, non-PDGF-binding aptamer further speaks to the E-AB sensor's selectivity. For the control, we employed a sequence (oligo 2) sharing 77% sequence identity with the PDGF-binding aptamer and designed to adopt a similar structure.[13] Sensors built using this control sequence do not exhibit any significant (<1%) change in signal when incubated for 30 min with 50 nM PDGF-BB in undiluted serum (Supporting Information S.I.4). A MB-labeled stem−loop DNA sequence (oligo 3) served as a second control sequence and, as expected, also fails to exhibit any significant signal increase under the same experimental conditions (data not shown).

To further ensure that the increase in current is not originating from processes such as sensor degradation, sensor regeneration is crucial. With a 4-min incubation in 10% sodium dodecyl sulfate followed by incubation in serum or buffer, we successfully recover ~95% of the original sensor signal (Supporting Information S.I.5), indicating the observed signal increase arises due to target binding rather than via some permanent modification of the electrode. This result suggests that the E-AB sensor is reusable, and indeed, we have used and regenerated such sensors up to 5 times before unacceptable degradation was observed. In undiluted serum, the sensor is stable for up to ~6 h without significant loss of performance. Of note, we have stored similar sensors constructed using a longer alkanethiol tether for 1 week in room-temperature serum without observing significant degradation.[27]

PDGF, a dimer composed of two different monomer types (A and B, which exhibit 60% sequence identity), occurs in three variants: PDGF-BB, -AB, and -AA. It has previously been shown that the aptamer employed here binds these variants with differing affinities,[22,28] an observation that the E-AB sensor recapitulates. In undiluted serum, at a protein concentration of 50 nM the variants BB, AB, and AA give rise to 52, 22, and 7% increases in E-AB current, respectively (Figure 5). Although the response for PDGF-AB is more similar to that for PDGF-BB than to that for PDGF-AA, the absolute current change upon aptamer binding between PDGF-AB and PDGF-BB is distinct and is comparable to results obtained with previously reported optical detection approaches.[29]

Given several recent reports detailing fluorescent or colorimetric sensors based on the same PDGF-binding aptamer, the results presented here also provide an opportunity for a direct comparison between the E-AB sensor and its analogous optical approaches. Such an assessment highlights the promise of the E-AB approach. For example, the detection limit of a recently reported colorimetric sensor based on the PDGF-induced aggregation of aptamer-coated gold nanoparticles is ~50 times poorer than that reported here, and no characterization of the sensor's ability to perform in contaminated samples has been

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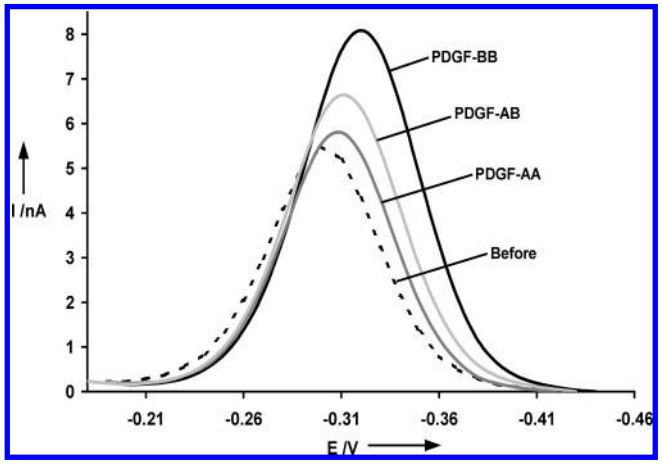

Figure 5. Differential response of the E-AB sensor to each of the three known PDGF variants. Shown are baseline-subtracted ac voltammograms for the E-AB sensor before use and after ~30-min incubation with 50 nM PDGF-AA, PDGF-AB, and PDGF-BB in undiluted blood serum.

described.[29] An alternative optical technique, based on binding-linked changes in fluorescence anisotropy, has a reported limit of detection of 220 pM.[21] The sensitivity of this approach, however, was similarly only reported for pure buffer and thus again no comparison with the E-AB sensor's selectivity is possible. Two fluorescence-based aptamer−beacon approaches have been described, however, for which selectivity data have been reported.[30] Of note, these approaches are direct optical analogues of the E-AB platform. The first, which works via the binding-induced segregation of a quencher-fluorophore pair, can detect as little as 110 pM concentration of the target protein when employed in pure buffer.[13] The selectivity of this approach is, however, relatively modest with only detection against "a nearly 1000-fold excess of serum-derived proteins" reported to date.[31] The second approach exhibits improved selectivity, albeit at the cost of a poorer detection limit; employing a binding-induced increase in pyrene-excimer fluorescence, this sensor exhibits a demonstrated detection limit of 2.5 nM in pure buffer but only 25 nM in culture media containing 10% calf serum.[30] It thus appears that the detection limit and selectivity of the E-AB sensor are several times lower and several thousand times higher, respectively, than those of the single most sensitive and the single most selective optical approaches reported to date. Moreover, the E-AB sensor combines this unprecedented sensitivity and selectivity in a single approach, rendering it possible for the first time to detect physiologically relevant PDGF levels directly in largely unprocessed clinical samples.

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CONCLUSIONS

In order to function in real-world applications, a sensor must achieve acceptable sensitivity, specificity, and selectivity. Here we show that an electrochemical E-AB sensor directed against the blood protein PDGF achieves physiologically relevant sensitivity and specificity and that it realizes these attributes even in the face of complex, contaminant-ridden clinical samples. In addition, the E-AB sensor features a number of other potential advantages as a sensing platform. As an electrochemical sensor, the E-AB platform does not require light sources, optics, high-voltage power supplies, or other potentially heavy, cumbersome equipment. The E-AB approach is also amenable to miniaturization and parallelization, potentially rendering it possible to simultaneously monitor multiple targets with a single-chip device. Last, because all of the E-AB components are strongly attached to the electrode surface, the approach is both single step and readily reusable. The E-AB approach thus appears well suited for convenient point-of-care diagnostics and the field monitoring of proteins and small molecule analytes.

ACKNOWLEDGMENT

This research was supported by the Center for Nanoscience Innovation for Defense (CNID) under DMEA90-02-2-0215, the Institute for Collaborative Biotechnologies (ICB) (DAAD19-03-D-0004), and Lawrence Livermore National Laboratory (LLNL) (URP-06-019).

SUPPORTING INFORMATION AVAILABLE

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Received for review August 25, 2006. Revised . Accepted October 17, 2006.

AC061592S