analytical chemistry

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Paper Electrochemical Device for Detection of DNA and Thrombin by Target-Induced Conformational Switching

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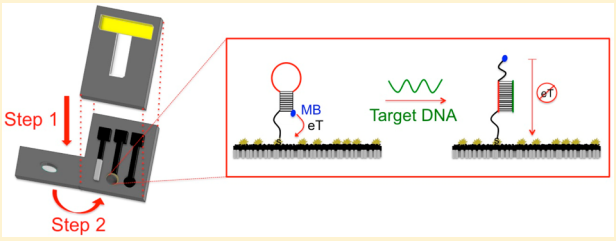
Supporting Information

ABSTRACT: Here, we report a strategy for the design of an inexpensive paper analytical device (PAD) for quantitative detection of oligonucleotides and proteins. Detection is based on the principle of target-induced conformational switching of an aptamer linked to an electrochemical label. This simple and robust method is well matched to the equally simple and robust characteristics of the PAD platform. The demonstrated limits of detection for DNA and thrombin are 30 nM and 16 nM, respectively, and the device-to-device reproducibility is better than ±10%. The PAD has a shelf life of at least 4 weeks, involves little user intervention, and requires a sample volume of just 20 μL.

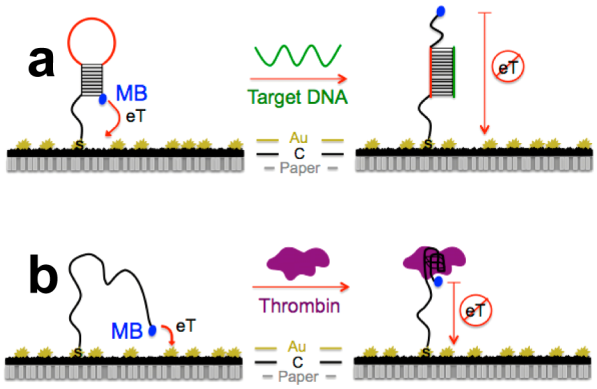


Paper analytical devices (PADs)[1,2] provide an inexpensive solution to medical and environmental diagnostic needs in both the developed and developing world.[3,4] Typically, analyte detection in PADs is through a color change with naked-eye observation,[3−7] fluorescence,[6,8] or electrochemical methods.[9−11] Of these, electrochemistry provides a good combination of simplicity, low power requirements, low limits of detection, and ease of quantitation.[1] Indeed, since the first report of electrochemical detection on a PAD by Henry and co-workers,[12] the popularity of this method has rapidly increased.[13,14]

In the present manuscript, we have adapted an electrochemical detection method, originally reported by Plaxco and co-workers,[15−17] to a paper fluidic platform. Surprisingly, the very simple, very inexpensive paper device provides figures of merit nearly identical to those obtained using traditional, macro-scale three-electrode electrochemical cells. This is all the more remarkable, because the devices are highly reproducible, have long shelf lives, and requires little user intervention.

Over the past decade, Plaxco and co-workers have demonstrated quantitative, wash-free electrochemical detection for a variety of analytes including small molecules,[18,19] proteins,[17,20,21] antibodies,[22,23] and DNA[15,24] in buffer, serum,[16,18] blood,[17,19] and soil using a method based on conformational switching of a surface-bound probe.[25] Others have also made important contributions to this family of sensors.[26−32] The general approach is illustrated in Scheme 1 for the two types of targets reported here: DNA and thrombin. As shown, the sensing mechanism is based on target-induced folding or unfolding of electrode-bound oligonucleotide probes that have a pendant redox reporter (typically methylene blue or ferrocene) at the distal end and a thiol at the proximal end for easy attachment to a gold electrode. When the analyte binds, the probe undergoes a conformational change that alters the location of the redox reporter relative to the electrode.

Scheme 1



Depending on the nature of this change, the redox reporter may move closer to the electrode (an "on" sensor) or further from the electrode (an "off" sensor, as shown in Scheme 1). This conformational change results in a change in faradaic current that is easily detected using either alternating current voltammetry (ACV) or square wave voltammetry (SWV). These types of experiments have been carried out using traditional three-electrode electrochemical cells[15,16,20] and in flow systems.[17,18]

EXPERIMENTAL SECTION

Chemicals and Materials. $AuCl_4^-$, KNO_3, and tris (2-carboxyethyl) phosphine hydrochloride (TCEP-HCl) were purchased from Sigma-Aldrich. Whatman grade 1 chromatog-

Received: April 20, 2014
Accepted: May 14, 2014
Published: May 28, 2014

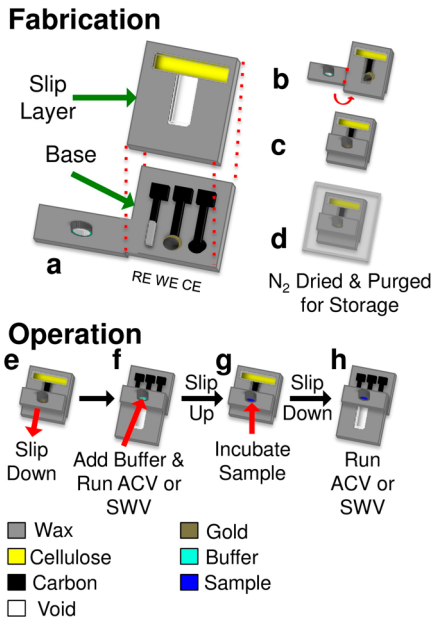
   dx.doi.org/10.1021/ac501438y | Anal. Chem. 2014, 86, 6166−6170

raphy paper, Tris-HCl buffer, H_2SO_4, KCl, $MgCl_2$, and NaCl were all purchased from Fisher Scientific. KH_2PO_4 was purchased from EM Science. Human α-thrombin was purchased from Haematologic Technologies Inc. (Essex Junction, VT). All oligonucleotides were purchased from Biosearch Technologies. 6-Mercapto-1-hexanol was obtained from Acros Organics. All chemicals were at least reagent grade. The potentiostat used for all experiments was a CHI Model 650c (CH Instruments, Austin, TX). The conductive carbon (CI-2042) and Ag/AgCl ink (CI-4002) were purchased from Engineered Conductive Materials (Delaware, Ohio), and a Xerox Color Cube 8570 printer was used to print solid wax on the chromatography paper. All solutions were prepared using deionized water (18.0 MΩ-cm, Milli-Q Gradient System, Millipore). All reagents were used as received without further purification. Experiments were conducted at room temperature (23 ± 2 °C).

RESULTS AND DISCUSSION

Esensor Fabrication. Scheme 2 illustrates the fabrication and operation of the paper electrochemical sensor, or "esensor"

Scheme 2



for short, used in this report. The device is based on the SlipChip concept originally reported by Ismagilov[33,34] and later adapted by us to the paper format as a SlipPAD.[35] As shown in Scheme 2a, the sensor consists of two pieces: a base layer and a slip layer. The base layer (2.2 cm wide and 2.4 cm long in the folded position) is fabricated using double-sided printing of a wax pattern onto chromatography paper.[36] A hole is punched in the paper flap on the base layer so that the working electrode (WE) will be exposed in the fully assembled device.

Three carbon electrodes are then printed onto the double wax-patterned paper. The WE and counter electrode (CE) are each 3 mm in diameter, and the base of the reference electrode (RE) is 1 mm wide. A layer of Ag/AgCl paste is then painted on top of the RE to ensure a stable potential. To simplify surface immobilization of probe DNA, Au is electroplated onto the carbon surface of the WE following literature procedures.[37,38] A scanning electron microscope image of the WE surface (Figure S1, Supporting Information) reveals micrometer-scale Au particles covering about 58% of the carbon surface. Finally, the probe DNA is incubated with the Au working electrode for an optimized period of time (discussed later), and then, defects in the probe monolayer are backfilled with 6-mercapto-1-hexanol to minimize nonspecific adsorption.[15]

Like the base layer, the slip layer (2.3 cm wide and 4.0 cm long) is also fabricated on a piece of chromatography paper such that a hydrophilic T-shaped section is surrounded by a hydrophobic wax frame. Next, the vertical section of the T is removed, leaving behind an open space. As shown in frames b–d of Scheme 2, the device is assembled by aligning the base and slip layers, folding the flap of the base layer over the top of the slip layer, and then edge laminating the entire device. As discussed later, in this sealed configuration, the device is stable for at least 4 weeks. Additional details regarding the device fabrication and characterization can be found in the Supporting Information.

Esensor Operation. The bottom row of Scheme 2 illustrates the operation of the paper esensor. First, the lamination is removed, and then, the channel is slipped down onto the three electrodes such that the horizontal paper section of the slip layer covers the three electrodes. Next, a small drop (~20 μL) of buffer is added to the hole in the slip layer, which wets the paper and brings the three electrodes into electrochemical contact so that a background ACV or SWV can be obtained.[10] The channel is then slipped up to expose the WE through the hole in the flap of the base layer, and the sample containing the target is introduced. After a predetermined incubation period, the channel is once again slipped into the measurement position and another ACV or SWV measurement is obtained.

ssDNA Esensor. For detection of single-stranded DNA (ssDNA), the electrode-bound receptor was a stem-loop structure, as shown in Scheme 1a. The stem-loop was functionalized on the 5′ end with a thiol group, to ensure stability on the electroactive gold working electrode, and on the 3′ end with methylene blue (MB), which functions as the electroactive reporter. The DNA sequence (see Supporting Information) of the stem-loop has previously been used by Plaxco and co-workers,[39] making it possible to directly compare the results of our paper esensor to their results obtained using a traditional electrochemical apparatus. The stem-loop was immobilized on the working electrode by immersing the latter in a 500 nM solution of the probe for 1 h, followed by backfilling with 6-mercapto-1-hexanol.[40] The optimum probe density (1.1×10^{12} molecules cm^{-2}) was determined experimentally (see Supporting Information) by maximizing the sensitivity of the esensor.[41,42]

The probe starts off in a stem-loop configuration, which positions the MB redox reporter in close proximity to the WE (Scheme 1a), thereby permitting efficient electron transfer. Upon binding to the ssDNA target, the stem-loop unfolds, the MB reporter moves away from the electrode, and there is a corresponding decrease in faradaic current. The level of signal suppression was evaluated by comparing the peak current in ACVs before and after addition of the ssDNA target. The data in Figure 1a shows that the initial peak current of 0.95 μA decreases by 45% after addition of 10.0 μM ssDNA. The average signal suppression and standard deviation, measured using five independently fabricated devices, are 51% and 4.6%, respectively (Figure 1b) for 10.0 μM target ssDNA. This value

dx.doi.org/10.1021/ac501438y | Anal. Chem. 2014, 86, 6166–6170

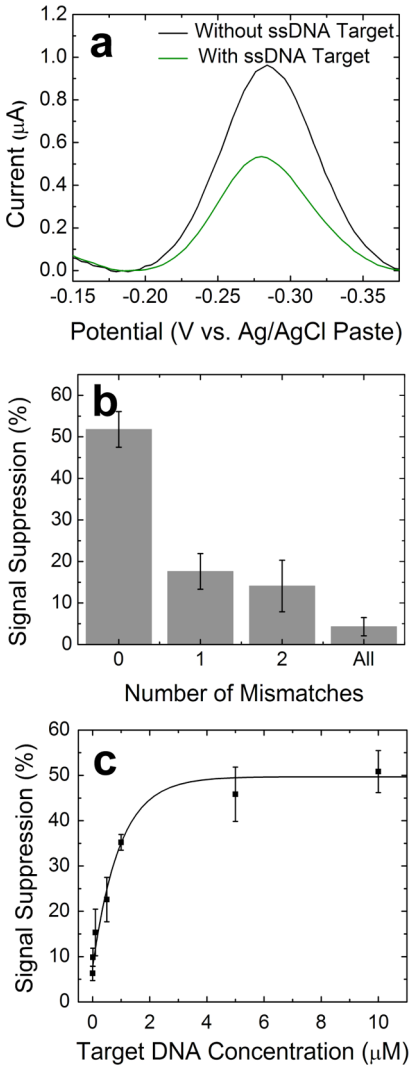


Figure 1. Detection of ssDNA using the device shown in Scheme 2 and the conformational switching approach shown in Scheme 1a. (a) Baseline-subtracted ACVs before and after addition of target ssDNA. (b) Histogram showing the percentage signal suppression for different numbers of base mismatches in the target. (c) Dose–response curve for ssDNA detection. The LOD is 30 nM.

can be compared with that obtained by Plaxco and co-workers (~50%) using the same stem-loop probe and ssDNA target, but a traditional three-electrode electrochemical cell.[39]

We also examined the sensitivity of the esensor to targets having one and two mismatched base pairs, as well as to a completely mismatched sequence, and the results (Figure 1b) indicated current suppressions of 18%, 14%, and 4.3%, respectively. The decreasing trend of signal suppression versus number of mismatches is consistent with the expectation that base pair mismatching destabilizes the duplex, which results in a smaller change in faradaic current.[43]

Figure 1c shows a dose–response curve for the paper DNA esensor upon exposure to the fully complementary target sequence. Each point in this plot represents the average determined using at least three paper esensors, and the error bars denote the associated standard deviations. A limit of detection (LOD) of 30 nM was determined from this plot by following a literature procedure wherein the LOD is equal to the blank signal plus 1.645 times the standard deviation of the lowest ssDNA concentration.[44]

Thrombin Esensor. To demonstrate the broad scope of this paper esensor design, we performed an assay for thrombin. In this case, the thrombin aptamer, which has been reported previously,[39] was immobilized on the Au working electrode by exposure to a 100 μM solution for 12 h, followed by backfilling with 6-mercapto-1-hexanol. The thrombin aptamer (Scheme 1b) is a dynamic single DNA strand that allows the MB redox reporter to approach the working electrode sufficiently often that a significant SWV signal is observed. However, upon binding to thrombin, the aptamer folds into a configuration that sequesters MB and, hence, reduces its access to the electrode surface and lowers the observed current. As for the previously discussed DNA target, the percentage change in faradaic current is directly related to the concentration of thrombin in the sample.

Figure 2a shows typical SWV results obtained for the esensor before and after exposure to 2.0 μM thrombin for 3 h. The

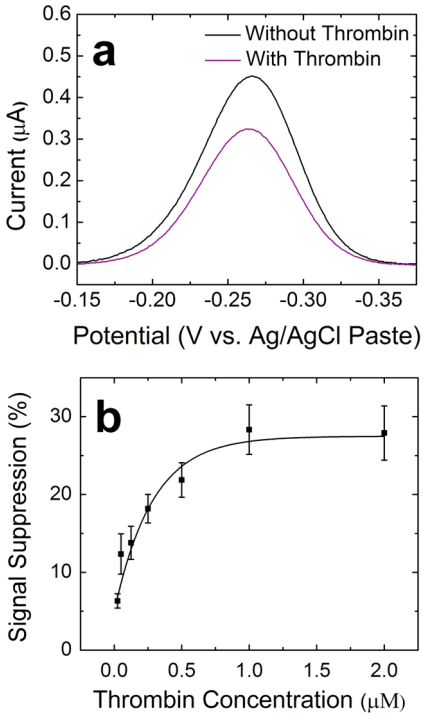


Figure 2. Detection of thrombin using the device shown in Scheme 2 and the conformational switching approach shown in Scheme 1b. (a) SWV before and after addition of thrombin. (b) Dose–response curve for detection of thrombin. The LOD is 16 nM.

initial peak current of 0.45 μA decreases by 29% after addition of thrombin. The average signal suppression and standard deviation for 2.0 μM of thrombin were determined to be 28% and 3.5%, respectively (Figure 2b), using three independently fabricated devices.

Figure 2b shows a dose–response curve for detection of thrombin with the paper esensor. Each point represents the average determined using three devices, and the error bars denote the associated standard deviations. The LOD, determined from this plot, is 16 nM.[44] This value compares favorably to previous results reported by Plaxco and co-workers.[45] They used a traditional three-electrode cell configuration and achieved a dynamic range of between a few

dx.doi.org/10.1021/ac501438y | Anal. Chem. 2014, 86, 6166–6170

nanomolar and several hundred nanomolar for thrombin detection in serum.

Esensor Stability. In previous reports of sensors based on conformational switching of aptamers, it was necessary for the probe to remain either in solution or in a dried sugar and bovine serum albumin matrix throughout the time required for its immobilization and testing.[40,46] This poses a problem for long-term storage of paper devices, because wax-patterned chromatography paper slowly absorbs water, becomes soggy, and hence is destabilized. We attempted to dry paper esensors in air after immobilization of the aptamers. However, as shown in Figure 3a, this resulted in a 39% decrease in current upon

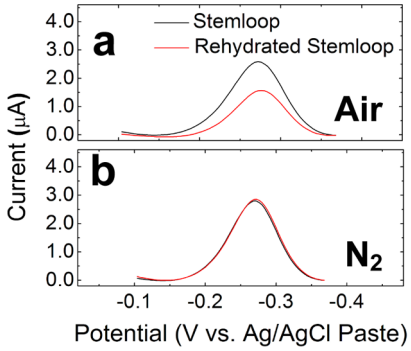


Figure 3. ACVs of the stem-loop (Scheme 1a) before and after drying with (a) air and (b) nitrogen.

rehydration. This observation probably arises from irreversible oxidation of the self-assembled alkanethiol monolayer and DNA,[47−49] which may lead to conformation changes and/or strand breakage.[46] However, if the aptamer is dried with nitrogen gas (Figure 3b), then the change in current is just 3%. Indeed, after 4 weeks of dry storage under nitrogen, the signal only decreases by 6%.

SUMMARY AND CONCLUSION

To summarize, we have reported a paper-based esensor that is based on the principle of target-induced conformational switching. This is a remarkably simple and robust approach to biosensing, and consequently, it is especially well-suited to the equally simple and robust characteristics of the type of PAD described here. Importantly, the paper esensor takes advantage of our previously reported SlipPAD design,[36] which provides a key enabling function: timed sample incubation directly on the WE. This function is not available in the types of continuous flow paper-based sensors that dominate the PAD field. The paper esensor design is easily adaptable to mass production, and the chemistry itself is sufficiently stable that the packaged esensor has a shelf life of at least 4 weeks. Moreover, the esensor is readily adaptable to other types of redox beacons, and hence, it seems likely that multiplexing of two or more sensors on a single device will be possible. Expanding the scope of the paper esensor design to include other targets and more realistic sample matrixes and further simplifying the design to eliminate the need for Au electrodeposition are our current areas of focus. The results of these experiments will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures including details of the device fabrication, detailed protocol for immobilization of probe and aptamer for ssDNA and thrombin sensors, all DNA sequences, parameters used for electrochemical methods, and gold working electrode characterization. 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.

ACKNOWLEDGMENTS

This project is sponsored by the Department of the Defense, Defense Threat Reduction Agency (contract number HDTRA-1-13-1-0031). R.M.C. thanks the Robert A. Welch Foundation (Grant F-0032) for sustained research support. J.C.C. thanks the NASA Harriett G. Jenkins Graduate Fellowship Program, a NASA Office of Education Minority University Research and Education Program (MUREP). We also thank Prof. Kevin Plaxco and Di Kang for providing DNA samples and for helpful discussions.

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